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T. Wallace
T. WALLACE
Certifying Officer



Submitted herewith for filing is the patent application of

Inventor: BEKA SOLOMON ET AL.

IMMUNIZATION AGAINST AMYLOID PLAQUES USING DISPLAY TECHNOLOGY

Enclosed are:

- ☒ 27 sheets of informal drawing(s).
- ☒ An assignment of the invention to RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD.
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Mark M. Friedman
Reg No. 33,883

Mark M. Friedman
DR. MARK FRIEDMAN LTD.
C/O Anthony Castorina
2001 Jefferson Davis Highway
Suite 207
Arlington, Virginia 22202

APPLICATION FOR PATENT

5

Inventors: Beka Solomon and Dan Frenkel

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IMMUNIZATION AGAINST AMYLOID PLAQUES
USING DISPLAY TECHNOLOGY

15

This is a continuation-in-part of U.S. Provisional Patent
20 Application No. 60/152,417, filed September 3, 1999.

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to methods, agents and compositions
25 for treating plaque-forming diseases, including, but not limited to,
Alzheimer's disease. More particularly, these methods involve the use of
(i) plaque derived antigens cloned and displayed on the surface of a
display vehicle for *in vivo* elicitation of antibodies capable of preventing
plaque formation and of disaggregating existing plaques; and (ii)
30 antibodies raised against plaque derived antigens, cloned and displayed

on a display vehicle, and which are capable of preventing plaque formation and of disaggregating existing plaques. The present invention further relates to a method of targeting a display vehicle to the brain of an animal, including man.

5 *Alzheimer's disease - clinical overview:*

Alzheimer's disease (AD) is a progressive disease resulting in senile dementia. Broadly speaking, the disease falls into two categories: late onset, which occurs in old age (typically above 65 years) and early onset, which develops well before the senile period, e.g., between 35 and 10 60 years. In both types of the disease, the pathology is similar, but the abnormalities tend to be more severe and widespread in cases beginning at an earlier age. The disease is characterized by two types of lesions in the brain, senile plaques and neurofibrillary tangles. Senile plaques are areas of disorganized neurofibrils up to 150 nm across with extracellular 15 amyloid deposits at the center, visible by microscopic analysis of sections of brain tissue. Neurofibrillary tangles are intracellular deposits of tau protein consisting of two filaments twisted about each other in pairs.

Senile plaques and other amyloid plaques:

The principal constituent of the senile plaques is a peptide termed A β or beta-amyloid peptide (β AP). The amyloid beta peptide is an internal fragment of 39-43 amino acids of a precursor protein termed amyloid precursor protein (APP). Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease (See, e.g., Goate *et al.*, Nature 349,704, 1991, valine⁷¹⁷ to isoleucine; Chartier Harlan *et al.* Nature 353, 844, 1991, valine⁷¹⁷ to glycine; Murrell *et al.*, Science 254, 97, 1991, valine⁷¹⁷ to phenylalanine; Mullan *et al.*, Nature Genet. 1, 345, 1992, a double mutation ,changing lysine⁵⁹⁵ -methionine⁵⁹⁶ to asparagine⁵⁹⁵-leucine⁵⁹⁶).

Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to beta-amyloid, particularly processing of APP to increased amounts of the long form of beta-amyloid (i.e., A β 1-42 and A β 1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form beta-amyloid (see Hardy, TINS 20, 154, 1997). These observations indicate that beta-

amyloid, and particularly its long form, is a causative element in Alzheimer's disease.

Amyloid deposits comprise a peptide aggregated to an insoluble mass. The nature of the peptide varies in different diseases but in most cases, the aggregate has a beta-pleated sheet structure and stains with Congo Red dye. In addition to Alzheimer's disease (AD), both late and early onset, other diseases characterized by amyloid deposits are, for example, SAA amyloidosis, hereditary Icelandic syndrome, multiple myeloma, and spongiform encephalopathies, including mad cow disease, Creutzfeldt Jakob disease, sheep scrapie, and mink spongiform encephalopathy (see, for example, Weissmann *et al.*, *Curr. Opin. Neurobiol.* 7, 695-700, 1997; Smits *et al.*, *Veterinary Quarterly* 19, 101-105, 1997; Nathanson *et al.*, *Am. J. Epidemiol.* 145, 959-969, 1997). The peptides forming the aggregates in these other diseases are serum amyloid A, cystatin C and IgG kappa light chain, respectively, for the first three, and prion protein for the others.

Other peptides or proteins with evidence of self aggregation are also known, such as, but not limited to, amylin (Young AA. *et al.*, 1994, *FEBS Lett.* 343(3);237-41); bombesin, caerulein, cholecystokinin

octapeptide, eledoisin, gastrin-related pentapeptide, gastrin tetrapeptide, somatostatin (reduced), substance P; and peptide, luteinizing hormone releasing hormone, somatostatin N-Tyr (Banks and Kastin, Prog Brain Res., 91:139-4, 1992).

5 ***Treatment:***

U.S. Pat. No. 5,688,561 to Solomon teaches methods of identifying monoclonal antibodies effective in disaggregating protein aggregates and preventing aggregation of such proteins. Specifically, U.S. Pat. No. 5,688,561 demonstrates anti-beta-amyloid monoclonal
10 antibodies effective in disaggregating beta-amyloid plaques and preventing beta-amyloid plaque formation *in vitro*. U.S. Pat. No. 5,688,561 stipulates the *in vivo* use of such antibodies to prevent plaque formation by aggregation of beta-amyloid or to disaggregate beta-amyloid plaques which have already formed. These teachings do not,
15 however, identify an epitope to be employed to generate such antibodies. In addition, these teachings do not provide means with which to enable the penetration of such antibodies into the brain through the blood brain barrier (BBB). Furthermore, this patent fails to teach the use of phage
display technology as a delivery method for antigens or antibodies. Yet

furthermore, no experimental results demonstrating the *in vivo* effectiveness of such antibodies are demonstrated by U.S. Pat. No. 5,688,561.

EP 526511 by McMichael teaches administration of homeopathic
5 dosages (less than or equal to 10^{-2} mg/day) of beta-amyloid to patients with pre-established AD. In a typical human with about 5 liters of plasma, even the upper limit of this dosage would be expected to generate a concentration of no more than 2 pg/ml. The normal
10 concentration of beta-amyloid in human plasma is typically in the range of 50-200 pg/ml (Seubert *et al.*, Nature 359, 325-327 1992). Because this proposed dosage would barely alter the level of endogenous circulating beta-amyloid and because EP 526511 does not recommend the use of an adjuvant, it seems implausible that any therapeutic benefit would result therefrom.

15 PCT/US98/25386 by Schenk and a Nature paper by Schenk *et al.* (Nature, 400:173-177, 1999) teach administration of beta-amyloid immunogens to a patient in order to generate antibodies to prevent formation of plaques or dissolve existing plaques. According to Schenk,
50 to 100 mg of antigen are required, 1 to 10 mg if an adjuvant is

employed. These teachings also stipulate that a similar effect may be achieved by direct administration of antibodies against beta-amyloid, in both cases disregarding the blood brain barrier which, under normal circumstances, prevents the penetration of antibodies into the brain.

5 It is also important to note that these teachings are typically restricted to the use of "...any of the naturally occurring forms of beta-amyloid peptide, and particularly the human forms (i.e., A β 39, A β 40, A β 41, A β 42 or A β 43)" or "...longer polypeptides that include, for example, a beta-amyloid peptide, active fragment or analog together with other
10 amino acids", or "multimers of monomeric immunogenic agents".

These teachings ignore, however, earlier data teaching that the first 28 amino acids of beta-amyloid are sufficient to elicit antibodies which both disaggregate and inhibit aggregation of beta-amyloid plaques *in vitro* (Hanan and Solomon, Amyloid: Int. J. Exp. Clin. Invest.
15 3:130-133, 1996; Solomon *et al.*, Proc. Natl. Acad. Sci. U.S.A. 93:452-455, 1996; Solomon *et al.*, Proc. Natl. Acad. Sci. U.S.A. 94:4109-4112, 1997).

Schenk and Schenk *et al.* both fail to teach the use of the N-terminal epitope of beta-amyloid plaques which is known to be a

sequential epitope composed of only four amino acid residues (EFRH, SEQ ID NO:1) located at positions 3-6 of the beta-amyloid peptide (Frenkel D., J. Neuroimmunol., 88:85-90,1998). Antibodies against this epitope have subsequently been shown to disaggregate beta-amyloid
5 fibrils, restore beta-amyloid plaques solubilization and prevent neurotoxic effects on PC 12 cells (Solomon, B. *et al.*, Proc. Natl. Acad. Sci. USA. 94:4109-4112, 1997; and Solomon, B., *et al.*, Proc. Natl. Acad. Sci. USA., 93:452-455,1996).

This epitope has been independently confirmed as the epitope
10 bound by anti-aggregating antibodies using random combinatorial hexapeptide phage display (Frenkel and Solomon, J. of Neuroimmunol. 88:85-90, 1998).

The EFRH (SEQ ID NO:1) epitope is available for antibody binding when beta-amyloid peptide is either in solution or in aggregates.
15 Blocking of this epitope by a monoclonal antibody prevents self-aggregation and enables resolubilization of already formed aggregates.

These findings suggest that the teachings of Schenk and colleagues are inefficient at best. Since, as has already been mentioned
hereinabove, the normal concentration of beta-amyloid in human serum

is 50-200 pg/ml, immunization with that peptide could be expected to produce either low antibody titers or high toxicity if strong adjuvants are used and as such it is not applicable for therapy. Indeed, in order to achieve significant serum titers of antibody against beta-amyloid a series of 11 monthly injections was required (Schenk *et al.*, Nature, 400:173-177, 1999). The degree to which these serum titers will persist over time is not yet known, and this point is especially crucial with respect to early onset Alzheimer's disease.

Schenk and colleagues further teach that an immunogenic peptide such as beta-amyloid may be displayed upon the surface of a virus or bacteria. However, they fail to teach use of an antigen so displayed to effect immunization. No mention is made of defining an epitope in this context and no experimental data is provided either. In addition, delivery of antibody displayed on a display vehicle is not taught by Schenk or Schenk *et al.* altogether.

Collectively, the prior art fails to teach means with which an effective titer of anti-aggregation antibodies can be generated *in vivo* in a short time and/or be introduced into the brains of patients suffering a

plaque-forming diseases. In addition, the persistence of titers generated via prior art teachings has not been established.

There is thus a widely recognized need for, and it would be highly advantageous to have, effective means of disaggregating amyloid plaques *in vivo* which would have lasting effect, high efficiency, rapid onset, no adverse effect on the treated subject and which is readily amenable to large scale production.

10 SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a method of treating a plaque forming disease comprising the steps of (a) displaying a polypeptide on a display vehicle, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein; and (b) introducing the display vehicle into a body of a recipient so as to elicit the antibodies capable of disaggregating the

aggregating protein and/or of preventing aggregation of the aggregating protein.

According to another aspect of the present invention there is provided an agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

According to yet another aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting an effective amount of antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, the pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

According to still another aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease, the method comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, such that when the display vehicle propagates the polypeptide is displayed by the display vehicle.

According to an additional aspect of the present invention there is provided a method of treating a plaque forming disease comprising the steps of (a) displaying a polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the binding capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein; and (b) introducing the display vehicle into a body of a recipient

so as to disaggregate the aggregating protein and/or prevent its aggregation.

According to still an additional aspect of the present invention there is provided a method of introducing a display vehicle lacking an engineered targeting moiety into a brain of a recipient, the method
5 comprising the step of administering the display vehicle intranasally to the recipient.

According to further features in preferred embodiments of the invention described below, the step of introducing the display vehicle
10 into the body of the recipient so as to disaggregate the aggregating protein is effected through an olfactory system of the recipient.

According to yet another additional aspect of the present invention there is provided an agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide representing at
15 least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of the aggregating protein.

According to yet an additional aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, the pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

According to still an additional aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding at least an immunological portion of an antibody capable of binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating

the aggregating protein and/or of preventing aggregation of the aggregating protein.

According to further features in preferred embodiments of the invention described below, the plaque forming disease is selected from
5 the group consisting of early onset *Alzheimer's* disease, late onset *Alzheimer's* disease, presymptomatic *Alzheimer's* disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

According to still further features in the described preferred
10 embodiments, the aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

According to additional further features in preferred embodiments of the invention, the display vehicle is selected from the group consisting
15 of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

According to still further features in the described preferred embodiments, the display vehicle is a virus.

According to still further features in the described preferred embodiments, the display vehicle is a bacteriophage.

According to still further features in the described preferred embodiments, the display vehicle is a filamentous bacteriophage.

5 According to further features in described preferred of the invention, the bacteriophage display vehicle is capable of propagating within bacterial flora of the host.

According to further features in described preferred embodiments of the invention, the bacteriophage display vehicle is capable of
10 propagating within *E. coli*.

According to still further features in the described preferred embodiments the bacteriophage display vehicle is fd.

According to further features in described preferred embodiments of the invention, the display vehicle is incapable of propagation *in vivo*.

15 According to still further features in the described preferred
— embodiments, a triple dose of 10^{10} units of the chosen display vehicle induces an antibody titer of at least 1:50,000 within 30 days of administration, as measured by ELISA. The present invention
successfully addresses the shortcomings of the presently known

configurations by providing methods, agents, and pharmaceutical compositions for quickly and reliably preventing or reversing the progression of a plaque forming disease. The invention further includes methods for preparing display vehicles for use as agents, or as part of
5 pharmaceutical compositions, associated with prevention or reversal of plaque forming diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the
15 cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show details of the invention in more detail than is necessary for a fundamental
— understanding of the invention, the description taken with the drawings

making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1a is a schematic depiction of an IgM antibody.

5 FIG. 1b is a photograph of an ethidium bromide stained 1.5 % agarose gel showing cDNA fragments of the heavy and the light chains of IgM508. Lane 1: Kb (Ladder); Lanes 2 and 3 V_H and V_L fragments, respectively, as indicated by arrows.

10 FIG. 1c is a photograph of an ethidium bromide stained 1.5 % agarose gel showing scFv DNA fragment derived from antibody IgM 508. Lane 1: Kb (Ladder); Lane 2: scFv 508 DNA (750 bp).

FIG. 1d is a schematic depiction of filamentous phage displaying an scFv.

FIG. 1e is a schematic depiction of a soluble scFv.

15 FIG. 2 is a physical map of plasmid pCC-508F which is used for the production of scFv-508-CBD fusion protein (also referred to herein as 508(Fv)-CBD) under control of lac promoter. Amp res - a gene encoding β -lactamase; V_H and V_L - sequences coding for the variable domains of the heavy and light chains of scFv-508, respectively; Lin - a

gene coding for a (Gly₄Ser)₃ (SEQ ID NO:2) linker present between the variable domains V_H and V_L. Restriction sites and positions thereof are also shown.

FIG. 3 is a physical map of plasmid pFEEKCA-508 which is used according to the present invention for cytoplasmic expression of the scFv-508-CBD fusion protein under the control of a T₇-promoter. Amp^r - a gene encoding β -lactamase; V_H and V_L - sequences coding for the variable domains of the heavy and light chains of scFv-508, respectively; Lin - a gene coding for a (Gly₄Ser)₃ (SEQ ID NO:2) linker present between the variable domains V_H and V_L. T₇-promoter and T₇ term - T₇ promoter and T₇ terminator sequences, respectively. Restriction sites and positions thereof are also shown.

FIG. 4 shows an analysis of β AP binding by antibody 508(Fv)-CBD in an ELISA assay. The analyzed antibodies were added to β AP coated wells. Bound antibodies were detected with HRP conjugated secondary antibodies. The parental 508 IgM antibody was used as a positive control. The unrelated anti- β -galactosidase antibody Gal6(Fv)-CBD was used as a negative control.

FIG. 5 shows PCR analysis of phage DNA inserts. DNA isolated from pCC-508(Fv), lane 2, and pCC-Gal6(Fv), Lane 3, were PCR amplified and separated on a 1.5 % agarose gel. Ethidium bromide staining and UV illumination were used to visualize the bands. Lane 1 contains a DNA size marker. The arrow marks the position of an intact scFv migrating at about 750 bp.

FIG. 6 demonstrates expression and purification of 508(Fv)-CBD. 5-10 μ g protein were loaded in each lane of a 14 % SDS polyacrylamide gel. Proteins were visualized by Coomassie brilliant blue staining. The arrow marks the position of the scFv-CBD fusion protein. Lane 1 - total cell extract from non-induced BL21(DE3) cells carrying 508((Fv)-CBD expression vector. Lane 2 - total cell extract from BL21(DE3) cells carrying 508((Fv)-CBD expression vector induced for 3 hours with IPTG. Lane 3 - washed, solubilized and reduced inclusion bodies that were used in refolding. Lane 4 - protein that did not bind to cellulose during cellulose-assisted refolding. Lane 5 - protein washed away from cellulose with TBS. Lane 6 - protein washed away from crystalline cellulose with distilled water. Lane 7 - soluble 508(Fv)-CBD recovered from cellulose by high-pH elution and neutralization.

FIG. 7 demonstrates the stability of 508(Fv)-CBD. Purified 508(Fv)-CBD protein was stored at 4 °C for one day (dark squares) or one week (dark circles), and then analyzed for β AP binding in an ELISA assay, as described in the legend to Figure 4. The unrelated antibody Gal6(Fv)-CBD served as a negative control (open squares).

FIG. 8 demonstrates quantitation of 508(Fv) mutants affinity-enrichment by PCR and DNA restriction analysis. The DNA of 19 508(Fv)-mutant micro library clones before (Figure 8a) and of 11 clones picked up after one cycle of affinity selection (Figure 8b) were analyzed. The DNA was digested with *PvuI* and separated on a 1.5 % agarose gel. A non-mutated scFv-CBD appears as an intact 1250 bp fragment (upper arrow). A mutated clone is indicated by the appearance of both 700 bp (middle arrow) and 550 bp (lower arrow) fragments. A DNA size marker is shown in lane 1.

FIG. 9 shows an analysis of β AP binding (Figure 9a) and stability (Figure 9b) of mutated 508(Fv) derivatives in an ELISA assay. The analyzed antibodies were added to β AP coated wells. Bound antibodies stored at 4 °C for one day or for one week were detected as described in the legend to Figure 7. 508(Fv) wild type (open squares), C96F (dark

squares), C96Y (dark circles), C96S (dark triangles). The unrelated anti- β -galactosidase antibody Gal6(Fv)-CBD was used as a negative control (open squares).

FIG. 10 shows an analysis of the specific inhibition of β AP binding by antibody 508F(Fv) in a competitive ELISA assay. The antibody was pre-incubated with varying concentrations of the competing peptides: β AP (acids 1-16 of SEQ ID NO:3) (dark squares) or the unrelated peptide WVLD (SEQ ID NO:4) (open squares), before being added to β AP coated wells. Bound antibodies were detected as described in the legend to Figure 7.

FIGs. 11a and 11b show nucleotide (SEQ ID NO:5) and deduced amino acid (SEQ ID NO:6) sequences of scFv 508F heavy chain (Figure 11a); and the linker and the variable region of the light chain (Figure 11b). The amino acid sequence is presented by a three-letter code; CDRs and the linker are underlined.

FIG. 12 demonstrates the prevention of β AP mediated toxic effect on PC12 cells by 508F(Fv). Cells were incubated with fibrillar β A alone, or with fibrillar β A that had been incubated with antibodies at different molar ratio of antibody/ β AP, as indicated. An 3-(4,5-dimethylthiazol-2-

yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to estimate cell survival.

FIG. 13 demonstrates the disaggregation of fibrillar β A by 508F(Fv). The fibrillar state of pre-formed β A fibrils were measured with or without incubation with antibodies at different molar ratio of antibody/ β AP, as indicated. The fluorescence of thioflavin-T (ThT) reagent in a ThT assay which is proportional to fibril β A was used to assess the fibril morphology.

FIGs. 14a-d demonstrate the detection of filamentous phage (f88-EFRH) in brain sections via immunofluorescence one day following a single dose applied intranasally. Appearance of filamentous phage in mouse olfactory bulb and hippocampus sections using fluorescent rabbit anti-phage antibody (Figures 14a and 14c, respectively) as is compare to an untreated mouse brain (Figures 14b and 14d, respectively). The sections were observed using a fluorescence microscope at a final magnification of x 10.

FIGs. 15a-d demonstrate the disappearance of filamentous phage (f88-EFRH) from mouse brain 28 days following a single intranasal administration. Disappearance of filamentous phage from mouse

olfactory bulb and hippocampus is demonstrated in sections of these organs using fluorescent rabbit anti-phage antibody (Figures 15a and 15c, respectively), as is compared to an untreated mouse brain (Figures 15b and 15d, respectively). The sections were observed using a fluorescence
5 microscope at a final magnification of $\times 10$.

FIGs. 16a-d show histology of mouse brain sections after phage f88-EFRH clearance. Brain sections of olfactory organ (Figure 16a) and hippocampus (Figure 16c) after 28 days following phage f88-EFRH administration were stained with hematoxylin and eosin, and compared to
10 sections of an untreated brain (Figures 16b and 16d, respectively). The stained sections were examined and photographed at a final magnification of $\times 40$.

FIGs. 17a-d show fluorescence detection of biotin of phage pCC-508F coupled to biotinylated β AP (acids 1-16 of SEQ ID NO:3) in mouse
15 brain sections following a single intranasal administration. Appearance of β AP (acids 1-16, SEQ ID NO:3) coupled to filamentous phage displaying scFv508F in mice olfactory bulb and hippocampus sections using streptavidin coupled to PE (Figures 17a and 17c, respectively) as is compare to an untreated mouse brain (Figures 17b and 17d, respectively).

The sections were observed using a fluorescence microscope at a final magnification of x 20.

FIGs. 18a-d show histology of mouse brain after phage pCC-508F coupled to biotinylated β AP (acids 1-16 of SEQ ID NO:3) administration. Olfactory organ (Figure 18a) and hippocampus (Figure 18b) sections one day following phage administration were stained with hematoxylin and eosin, and were compared to untreated mouse brain sections (Figures 18c and 18d, respectively). The stained sections were examined and photographed at a final magnification of x 40.

FIG 19 is a diagram of immunization schedule with filamentous phage displaying the EFRH (SEQ ID NO:1) epitope of β -amyloid peptide.

FIGs. 20a and 20b show immunization with f3 filamentous phage displaying EFRH (SEQ ID NO:1) epitope of β -amyloid peptide as a fusion of phage glycoprotein III (gpIII). Serum IgG titer of different bleeds from mice immunized with the EFRH-phage according to the schedule of Figure 19 against wild type filamentous phage coat proteins (Figure 20a) and the N-terminal epitope (acids 1-16, SEQ ID NO:3) of β -amyloid (Figure 20b).

FIG. 21 demonstrates long lasting immunization with f3 filamentous phage. Serum IgG titer of different bleeds from mice immunized with EFRH-phage against wild type filamentous phage coat proteins and the N-terminal (acids 1-16, SEQ ID NO:3) of β -amyloid.

5 FIG. 22 show binding of anti-aggregating β AP monoclonal antibody (mAb 10D5) to peptide-presenting phage selected from an f88 phage library. Unrelated mAb 5.5 raised against acetylcholine receptor was used as a negative control. Antibodies were added to phage-coated wells and ELISA was used to detect binding.

10 FIG. 23 show binding of anti-aggregating β AP mAb (10D5) to a YYEFRH (SEQ ID NO:7)-phage and VHEPHEFRHVALNPV (SEQ ID NO:8)-phage. Antibody in concentration of 1 μ g/ml was added to phage-coated wells and binding was analyzed by ELISA. Filamentous phage without insert was used as a control.

15 FIGs. 24a-b show immunization with f88 filamentous phage displaying EFRH (SEQ ID NO:1) epitope of β -amyloid peptide as a fusion of phage glycoprotein VIII (gpVIII). Serum IgG titer of different bleeds from mice immunized with EFRH-phage against wild type

filamentous phage coat proteins (Figure 24a) and the N-terminal epitope (acids 1-16, SEQ ID NO:3) of β -amyloid peptide (Figure 24b).

FIG. 25 shows inhibition of serum of an immunized mice in binding to β AP by synthetic peptides derived from the N-terminal of β -amyloid peptide. The assay was done with 1:3000 dilution of serum after a third immunization with f88-EFRH reacted with the various peptides in various concentrations per well, as indicated. The peptide WVLD (SEQ ID NO:4) was used as a negative control.

FIG. 26 demonstrates prevention of β AP mediated toxic effect on PC12 cells by serum antibodies raised against f88-EFRH-phage. Cells were incubated with fibrillar β A alone, or with fibrillar β A that has been incubated with serum from the third bleeding at different concentration. The negative control was serum from a non-immunized mouse. The MTT assay was used to estimate cell survival.

FIG. 27 demonstrates interference with fibrillar β -amyloid formation by serum antibodies raised against the f88-EFRH-phage. Estimation of the fluorescence of ThT which correlates with the amount of fibrillar β -amyloid formed after incubation for a week at 37 °C in the presence of serum samples diluted as indicated. The negative control was

serum from a non-immunized mouse. The positive control was without serum. Fibril formation was measured by the ThT assay.

5 DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of methods, pharmaceutical agents and compositions which can be used for treating plaque-forming diseases, including, but not limited to, Alzheimer's disease. Specifically the present invention can be used to (i) induce active immunity to plaque
10 derived antigens in a recipient by immunizing with at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease on a display vehicle, so that antibodies elicited in response to immunization are capable of preventing plaque formation and/or of disaggregating existing plaques; and (ii) induce passive
15 immunity by administering at least an immunological portion of an antibody which can bind to at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease, raised against plaque derived antigens, cloned and displayed on a display vehicle, capable of preventing plaque formation and of disaggregating

existing plaques. This passive immunity may be of exceptionally long duration if the display vehicle employed is capable of replicating within the recipient. The present invention further relates to a method of targeting a display vehicle to the brain of an animal, including man, so
5 that plaques present in the brain, such as beta amyloid plaques in brains of Alzheimer's disease patients, may be disaggregated.

The principles and operation of immunization according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

10 Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description. The invention is capable of other embodiments or of being practiced or carried out in
15 various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

While reducing one aspect of the present invention to practice, as is further exemplified in the Examples section that follows, antigens

derived from beta amyloid peptide were displayed on the surface of a filamentous phage which was used for immunization of experimental animals. All of the peptides employed contained the EFRH epitope (SEQ ID NO:1, residues 3-6, SEQ ID NO:3) of beta amyloid peptide (SEQ ID NO:3). The epitope was presented as a fusion protein of fd phage coat glycoprotein III or VIII. Doses ranging from 10^{10} to 10^{12} phages per injection were employed on 8 week old female BALB/c mice. A typical immunization schedule included three injections at 14-day intervals, administered either intraperitoneally or intranasally.

During and after the immunization process, the antibody serum titer of subject mice was tested for the production of A β specific antibodies by enzyme linked immunosorbent assay (ELISA) as detailed in methods and materials hereinbelow. Serum titers were subsequently shown to persist for 11 months in response to a protocol including only 3 immunizations. While all tested epitopes containing EFRH produced a titer, displaying the epitope on the surface of a display vehicle produced far highest and unexpected titers. These high titers are believed to be a result of the great number of copies presented to the immune system

using this method, and this idea is supported by results of binding assays using controlled amounts of sera.

The anti-aggregating properties of the obtained polyclonal antibody raised against EFRH epitopes with respect to beta-amyloid fibril formation was measured by the ThT binding assay. Serum, at dilution of 1:10 and 1:100, disrupted formation of fibril structure of β -amyloid with extensive deterioration of fibril morphology, as indicated by a substantial decrease in ThT fluorescence. The unrelated serum used as control (serum from un-immunized mouse) did not significantly inhibit fibril formation.

The effect of the same serum on disruption of already formed β A fibril (the toxic form of β AP) was also determined. Serum of EFRH immunized mice incubated with pre-formed β A fibrils disrupted the fibril structure. The unrelated control antibody had no significant effect on fibril morphology. Together, these results confirm the ability of EFRH epitope presented by suitable display vehicles to evoke production of anti-aggregation antibodies which can inhibit or reverse the process of fibril formation.

Diluted serum produced according to this embodiment of the present invention prevented the neurotoxicity of beta amyloid peptide. This result implies potential clinical utility in preventing brain deterioration of patients suffering from amyloid plaque diseases.

5 Thus, according to one aspect of the present invention there is provided a method of treating a plaque forming disease. The method according to this aspect of the present invention is effected by displaying a polypeptide on a display vehicle, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in
10 the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, and introducing the display vehicle into a body of a recipient, so as to elicit the antibodies capable of disaggregating the aggregating protein and/or
15 of preventing aggregation of the aggregating protein.

According to a preferred embodiment of the present invention the display vehicle is selected such that less than 30 days following an introduction of a triple dose of 10^{10} units thereof to the recipient, a titer

of the antibodies in the recipient is above 1:50,000, as is determined by ELISA.

According to another aspect of the present there is provided an agent for treating a plaque forming disease. The agent according to this aspect of the present invention comprising a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

According to still another aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease. The composition according to this aspect of the present invention comprising an effective amount of a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting an effective amount of antibodies capable of disaggregating the aggregating

protein and/or of preventing aggregation of the aggregating protein, and a pharmaceutically acceptable carrier.

According to yet another aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease. The method according to this aspect of the present invention is effected by genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, such that when the display vehicle propagates the polypeptide is displayed by the display vehicle.

Use of beta amyloid peptide antigens in conjunction with adjuvants to effect immunization has previously been difficult due to a combination of high toxicity and low titers which result. Using prior art methods as a starting point, immunization of a mouse with a 16 amino acids peptide of beta-amyloid conjugated to KLH (SEQ ID NO:9) was

carried out. This immunization produced a low but measurable antibody titer against beta-amyloid.

While reducing another aspect of the present invention to practice, splenectomy of the immunized mouse facilitated preparation of IgM hybridoma 508 expressing scFvAb with specificity to beta-amyloid. RNA was subsequently extracted from this hybridoma and was employed for antibody cloning. IgM 508 hybridoma showed specific activity to A β in preventing its toxic affect on PC12 cells (Anavi, S. 1998, M. Sc. thesis from the department of Molecular Microbiology and Biotechnology of the Tel-Aviv University, Israel). V_H and V_L sequences of IgM 508 were cloned separately and linked using a commercially available vector to form a single chain antibody with anti-beta amyloid specificity. This single chain antibody was subsequently expressed as a fusion protein in a phage display library and clones with anti beta amyloid activity were selected for propagation in *E. coli*.

Further reduction to practice was demonstrated by determining the apparent binding constants of the purified antibody presenting phage to amyloid beta were measured by ELISA test, and half-maximal binding was obtained at an antibody concentration of 340 ng/ml, corresponding to

8x10⁻⁶ M. This result anticipate that the prepared single chain antibody will be effective under *in vivo* conditions. This phage was also able to disrupt already formed fibril structures confirming that the purified single chain antibody is biologically active, as suggested by the binding
5 constant determination.

Thus, according to an additional aspect of the present invention there is provided a method of treating a plaque forming disease. The method according to this aspect of the present invention is effected by displaying a polypeptide representing at least an immunological portion
10 of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the binding capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, and introducing the display vehicle into a body of a recipient so as to disaggregate the
15 aggregating protein and/or prevent its aggregation.

According to a preferred embodiment of the present invention, and as is further described hereinbelow and exemplified hereinunder in the Examples section, introducing the display vehicle into the body of the recipient so as to disaggregate the aggregating protein and/or prevent the

aggregation of the aggregating protein is effected through an olfactory system of the recipient.

According to yet an additional aspect of the present invention there is provided an agent for treating a plaque forming disease. The agent according to this aspect of the present invention comprising a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of the aggregating protein.

According to still an additional aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease. The composition according to this aspect of the present invention comprising an effective amount of a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the

immunological portion of the antibody being capable of disaggregating the aggregating protein, and a pharmaceutically acceptable carrier.

According to a further aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease. the method according to this aspect of the present invention is effected by genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding at least an immunological portion of an antibody capable of binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein.

For purposes of this specification and the accompanying claims the terms "patient", "subject" and "recipient" are used interchangeably. They include humans and other mammals which are the object of either prophylactic, experimental, or therapeutic treatment.

For purposes of this specification and the accompanying claims, the terms "beta amyloid peptide" is synonymous with " β -amyloid peptide", " β AP", " β A", and " $A\beta$ ". All of these terms refer to a plaque forming peptide derived from amyloid precursor protein.

As used herein in the specification and in the claims the term "disaggregating" refers to solubilization of aggregated proteins. Aggregated A β , for example, is a mixture of oligomers in which the monomeric units are held together by non covalent bonds.

5 For purposes of this specification and the accompanying claims the terms "comprising" refers to inclusion of one or more recited element but does not exclude other elements not specifically recited. For example, a composition that comprises A β peptide encompasses both an isolated A β peptide and A β peptide as a component of a larger
10 polypeptide sequence. Similarly, an immunological portion of an antibody may be included as a part of a larger of the antibody, say the entire antibody.

As used herein in the specification and in the claims section that follows, the term "treating" includes substantially inhibiting, slowing or
15 reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or substantially preventing the appearance of clinical symptoms of a disease.

As used herein in the specification and in the claims section that follows, the term "plaque forming disease" refers to diseases

characterized by formation of plaques by an aggregating protein (plaque forming peptide), such as, but not limited to, beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain or prion protein, in diseases such as, but not limited to, early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma or spongiform encephalopathy.

Because amyloid plaques associated with the diseases described hereinabove are located within the brain, any proposed treatment modality must demonstrate an ability to cross the blood brain barrier (BBB) as well as an ability to dissolve amyloid plaques. Normally, the average size of molecules capable of penetrating the BBB is approximately 2 kDa. Monoclonal antibodies are typically in the range 135-900 kDa. Therefore, future therapeutic use of antibodies in treating amyloid plaque diseases must rely on either reduction of their size concurrent with retention of activity, or on development of novel delivery strategies.

Small synthetic peptides consisting of antigen epitopes, such as the EFRH (SEQ ID NO:1) epitope of A β described hereinbelow, are in

general poor antigens and need to be coupled to a larger carrier. Even after coupling they may induce only a low affinity immune response. Injection of A β -KLH or A β -fibril leads to very slow immune response (Anavi, S., 1998, M. Sc. thesis from the Department of Molecular
5 Microbiology and Biotechnology of the Tel-Aviv University) and many efforts have been made to circumvent low affinity response, with limited success.

Since the pathological effects of amyloid beta fibril in AD patients are maintained only in the central nervous system (CNS), the capability
10 of highly specific mAbs in preventing Amyloid beta fibril toxicity *in vivo* tests is dependent on the permeability of the blood brain barrier (BBB) . In the progressive stage of AD, evidence shows alteration in the permeability of the BBB, which may lead to direct delivery of such antibody from the periphery to the CNS to disaggregate already formed
15 plaques and minimize further toxic effects (Schenk *et al.* , Nature 1999, 100:173-177). Preferred embodiments of the present invention include such direct delivery of scFv Ab presented on display vehicles to the brain across the blood brain barrier.

An increasing body of evidence shows that olfactory deficits and degenerative changes in the central olfactory pathways are affected early in the clinical course of AD. Moreover, the anatomic patterns involved in AD suggest that the olfactory pathway may be the initial stage in the
5 development of AD.

Olfactory receptor neurons are bipolar cells that reside in the epithelial lining of the nasal cavity. Their axons traverse the cribriform plate and project to the first synapse of the olfactory pathway in the olfactory pathway in the olfactory bulb of the brain. This configuration
10 makes them a highway by which viruses or other transported substances may gain access to the CNS across the BBB. In the early stages of AD the BBB may limit the entry of antibody circulating in the periphery to the CNS. In contrast A β anti-aggregating antibodies displayed on a phage surface have the potential not only be delivered directly to the
15 CNS by intranasal administration but also to prevent olfactory permanent damage by β A in the patients. As previously shown, intranasal administration (Mathison *et al.*, J. Drug Target, 1998) 5(6), 415-441; Chou *et al.*, Biopharm Drug Dispos. 1997) 18(4):335-46; *et al.*, Gene

Therapy 1995) 2:418-423) enables the direct entry of viruses and macromolecules into the CSF or CNS.

Use of olfactory receptor neurons as a point of delivery for an adenovirus vector to the brain is reported in the literature. This method
5 reportedly causes expression of a reporter gene in the brain for 12 days without apparent toxicity (Draghia *et al.*, Gene Therapy 2:418-423, 1995).

Thus, according to a preferred embodiment of the present invention, a vehicle displaying an immunological portion of an antibody
10 as herein described is delivered via this route to the brain.

As A β is produced continuously by cells in peripheral tissues which cross the blood brain barrier (BBB) leading to localized toxic effects in specific neuronal populations, intranasal administration of such a vehicle may also prevent the progression of the disease by minimizing
15 the amount of peripheral A β available to form plaques.

— The use of display vehicles such as filamentous phages as a drug delivery system to the CNS opens new horizons for therapeutic approaches for Alzheimer's disease, as well as for other

neurodegenerative diseases involving toxic extracellular aggregation of human peptides.

The display vehicle according to the present invention can be of any type including viral (e.g., bacteriophage, such as filamentous
 5 bacteriophage, fd, for example), bacterial and prion display vehicles. Thus, for example, the display vehicle can be a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion. According to a preferred embodiment of the present invention the display vehicle is capable of propagation in the recipient. Thus, for example, a
 10 bacteriophage display vehicle can be propagated in bacterial flora, such as *Escherichia coli* residing in the recipient's body. Alternatively, the display vehicle is an *in vivo* non-propagateable particle.

The phage or virus vehicle has promise as a targetable *in vivo* therapy approach. Although concerns about the potential infection of the
 15 natural intestinal flora (Delmastro *et al.*, Vaccine 1997, 15: 1276-1285; Willis *et al.*, Gene. 1993, 128:79-83; Poul *et al.*, J. Mol. Biol. 1999, 288, 203-211) have been expressed, UV inactivation of phage showed (Delmastro *et al.*, Vaccine 1997, 15: 1276-1285) that they are as
 immunogenic as their infective counterparts. Use of inactivated phage

may preclude incorporation of phage encoded transgenes into the nucleus for subsequent expression in host cells (Larocca *et al.*, 1998, Hum. Gene Ther. 9:2393-2399), an important practical consideration. Therefore, according to alternate preferred embodiments, the display vehicles
5 employed in the present invention may be either replicating or non-replicating.

Phage or virus display involves the expression of cDNA clones as fusion proteins with phage or virus coat proteins. If the cDNAs selected for expression encode antigens, the phage or virus may then be employed
10 as an antigen presenting vehicle, which can optionally replicate within a recipient.

As described above, according to preferred embodiments of the present invention, antigens displayed by a phage or virus may be used directly for vaccination, without antigen purification. In this case, the
15 bulk of the coat proteins serve to stimulate a general immune response because they are "non-self" with respect to the vaccinated subject. The antigen-coat protein fusion elicits a specific antibody against epitopes in the displayed cDNA gene product.

Antibody phage or virus display is accomplished, for example, by fusing the coding sequence of the antibody variable regions to a phage or virus coat protein. To this end, the variable (V) regions (V_H and V_L) mRNA isolated from antibody-producing cells is reverse-transcribed into cDNA, and heavy and light chains assembled randomly to encode single chain Fv (scFv). These cassettes are cloned directly into a suitable vector such as a phagemid vector for expression and display on the phage or virus surface. This linkage between antibody genotype and phenotype allows the enrichment of antigen specific phage or virus antibodies, using immobilized or labeled antigen. Phage or virus that display a relevant antibody will be retained on a surface coated with antigen, while non-adherent phages or viruses will be washed away. Bound phages or viruses can be recovered from the surface, re-infected into suitable host cells and re-grown for further enrichment and, eventually for binding analysis.

The success of antibody phage or virus display hinges on the combination of this display and enrichment method. Phage or virus antibody genes can be sequenced, mutated and screened to improve antigen binding.

It is possible to rearrange the genes which code for the various regions of an antibody molecule such that its specificity and affinity for an antigen are altered. The antibody can be maintained on the surface of the phage or virus for further manipulation or be released as soluble scFv (~25 kDa) fragment.

Since its invention at the beginning of the 1990's, antibody phage display has revolutionized the generation of monoclonal antibodies and their engineering. This is because phage display allows antibodies to be made completely *in vitro*, bypassing the immune system and the immunization procedure, and allowing *in vitro* tailoring of the affinity and specificity of the antibody. It is therefore anticipated that the most efficient new vaccine development strategies will employ this technology.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus such as antibiotic sensitivity. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of

antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

Viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell.

As used herein in the specification and in the claims section that follows, the term polypeptide refers to a stretch of amino acids covalently linked there amongst via peptide bonds. Different polypeptides have different functionalities according to the present invention. While according to one aspect a polypeptide is derived from an immunogen designed to induce an active immune response in a recipient, according to another aspect of the invention, a polypeptide is derived from an

antibody which results following the elicitation of an active immune response, in, for example, an animal, and which can serve to induce a passive immune response in the recipient. In both cases, however, the polypeptide is encoded by a polynucleotide according to any possible

5 codon usage.

As used herein the phrase "immune response" or its equivalent "immunological response" refers to the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against an

10 aggregating protein (plaque forming peptide) in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class

15 II MHC molecules, to activate antigen-specific $CD4^{+}$ T helper cells and/or $CD8^{+}$ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or other components of innate

immunity.

As used herein "active immunity" refers to any immunity conferred upon a subject by administration of an antigen.

As used herein "passive immunity" refers to any immunity conferred upon a subject without administration of an antigen. "Passive immunity" therefore includes, but is not limited to, administration of a replicating display vehicle which includes an immunological portion of an antibody presented on its surface to a recipient. Although replication of such a vehicle is active, the immune response is passive from the standpoint of the recipient.

10 For purposes of this specification and the accompanying claims the terms "epitope" and "antigenic determinant" are used interchangeably to refer to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein.

15 Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining

spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen. T-cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4 cells. T cells that recognize the epitope can be identified by *in vitro* assays that measure antigen-dependent proliferation, as determined by ^3H -thymidine incorporation by primed T cells in response to an epitope (Burke *et al.*, J. Inf. Dis. 170, 1110-19, 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges *et al.*, J. Immunol. 156, 3901-3910) or by cytokine secretion.

The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4^+ T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating IgG and T-cells from an immunized

syngeneic animal and measuring protective or therapeutic effect in a second subject.

As used herein and in the claims, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several
5 classes of structurally related proteins that function as part of the immune response of an animal or recipient, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins.

Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and
10 are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains.

15 In order to produce polyclonal antibodies, a host, such as a rabbit or goat, is immunized with the antigen or antigen fragment, generally with an adjuvant and, if necessary, coupled to a carrier. Antibodies to the antigen are subsequently collected from the sera of the host. The polyclonal antibody can be affinity purified against the antigen rendering

it monospecific. Previous experience has shown that standard production of polyclonal antibodies is not the method of choice for preparation of disaggregating antibodies for plaque forming peptides due to problems of poor titer and toxicity.

- 5 In order to produce monoclonal antibodies hyperimmunization of an appropriate donor, generally a mouse, with the antigen is undertaken. Isolation of splenic antibody producing cells is then carried out. These cells are fused to a cell characterized by immortality, such as a myeloma cell, to provide a fused cell hybrid (hybridoma) which can be maintained
- 10 in culture and which secretes the required monoclonal antibody. The cells are then be cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use. By definition, monoclonal antibodies are specific to a single epitope. Monoclonal antibodies often have lower affinity constants than polyclonal antibodies raised against
- 15 similar antigens for this reason.

— Monoclonal antibodies may also be produced ex-vivo by use of primary cultures of splenic cells or cell lines derived from spleen (Anavi, S., 1998. Locking the N-terminal of the Alzheimer β -amyloid peptide prevents the neurotoxicity in cell cultures, M. Sc. thesis. In order to

produce recombinant antibody (see generally Huston *et al*, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing B-lymphocytes of animals, or hybridoma are reverse-transcribed to obtain complementary DNAs (cDNAs). Antibody cDNA, which can be full length or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, Immunochemistry in Practice, Blackwell Scientific Publications, Oxford, 1982. The binding of antibodies to a solid support substrate is also well known in the art. See for a general discussion Harlow & Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory

Publications, New York, 1988 and Borrebaeck, Antibody Engineering - A Practical Guide, W.H. Freeman and Co., 1992.

As used herein and in the claims, the phrase "an immunological portion of an antibody" include an $F(ab')_2$ fragment of an antibody, an
5 Fab fragment of an antibody, an F_v fragment of an antibody, a heavy chain of an antibody, a light chain of an antibody, an unassociated mixture of a heavy chain and a light chain of an antibody, a heterodimer consisting of a heavy chain and a light chain of an antibody, a catalytic domain of a heavy chain of an antibody, a catalytic domain of a light
10 chain of an antibody, a variable fragment of a light chain of an antibody, a variable fragment of a heavy chain of an antibody, and a single chain variant of an antibody, which is also known as scFv. In addition, the term includes chimeric immunoglobulins which are the expression products of fused genes derived from different species, one of the species
15 can be a human, in which case a chimeric immunoglobulin is said to be humanized. Typically, an immunological portion of an antibody competes with the intact antibody from which it was derived for specific binding to an antigen.

Optionally, an antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

As used herein the terms "immunogenic agent" or "immunogen" or "antigen" are used interchangeably to describe a molecule capable of inducing an immunological response against itself on administration to a recipient, either alone, in conjunction with an adjuvant, or presented on a display vehicle.

As used herein the term "adjuvant" refers to a compound that, when administered in conjunction with an antigen, augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

A pharmaceutical preparation according to the present invention includes, as an active ingredient, a display vehicle displaying at least one epitope of an aggregating protein associated with plaque formation in a

plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein.

Alternatively, a pharmaceutical composition according to the present invention includes, as an active ingredient, a display vehicle displaying at

5 least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein.

The preparation according to the present invention can be
10 administered to an organism *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and
15 excipients. The purpose of a pharmaceutical composition is to facilitate
— administration of a compound to an organism.

Herein the term "active ingredient" refers to the preparation accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer a preparation in a local rather than systemic manner, for example, via injection of the preparation directly into the brain of a patient.

Pharmaceutical compositions of the present invention may be
5 manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using
10 one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the invention may be
15 formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally

contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to
5 characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such
10 as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should
15 be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for
use according to the present invention are conveniently delivered in the

form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic

solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired circulating antibody concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma or brain levels of antibodies which are sufficient to prevent aggregation or disaggregate existing aggregates (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Binding assays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains plasma levels above the MEC for 10-90 % of the time, preferable between 30-90 % and most preferably 50-90 %.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction,

the manner of administration, the judgment of the prescribing physician,
etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit,
5 which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a
10 form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an
15 approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as if further detailed above.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook *et al.*, (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical

Guide to Molecular Cloning", John Wiley & Sons, New York (1988);

Watson *et al.*, "Recombinant DNA", Scientific American Books, New York; Birren *et al.* (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York

5 (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites *et al.* (eds), "Basic and Clinical Immunology" (8th Edition),

10 Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517;

15 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell

Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak *et al.*, "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Reference is made to the following materials and methods, which were employed in experiments described in the following examples.

MATERIALS AND EXPERIMENTAL METHODS

The following materials and experimental methods were employed while reducing the present invention to practice as is further demonstrated in the Examples that follow:

General recombinant DNA and phage techniques:

Standard recombinant DNA techniques were performed essentially as described (Sambrook *et al.*, 1989). General protocols for antibody-phage display technology are from the Pharmacia Biotech (Uppsala, Sweden) Recombinant Phage Antibody System (RPAS).

Construction of 508 scFv on the phage display:

The 508 IgM hybridoma used as the source for antibody variable-region sequences was generated from splenocytes of a mouse that had been immunized with a peptide corresponding to the 16 amino terminal residues of β AP conjugated to keyhole limpet hemocyanin, used as a carrier. mRNA extraction, first strand cDNA synthesis, PCR amplification of variable heavy (V_H) and variable light (V_L) sequences, and assembly of scFv cassettes, were done according to protocols essentially as described (Pharmacia Biotech RPAS manual). Assembled 508 scFv DNA was digested with *Sfi*I and *Not*I, and 100 ng were ligated with 150 ng of vector DNA prepared by digestion of phagemid pCC-Gal6(Fv) (Berdichevsky Y *et al.*, J Immunol Methods., 31;228(1-2):151-62,1999) with *Sfi*I and *Not*I. This phage-display system is designed to express the scFv in frame fusion protein with cellulose binding domain

(CBD) derived from *Clostridium thermocellum* (Morag E *et al.* Appl Environ Microbiol., 61(5):1980-6, 1995). Ligated DNA was introduced into XL-1 Blue cells (Stratagene, La Jolla, CA) by transformation and transformants were plated onto 2 X YT Agar plates containing 100 µg/ml
 5 ampicillin and 1 % glucose for overnight growth at 37 °C.

Selection of β -amyloid binding scFv-CBD fusion proteins:

Individual clones were picked and grown, each in 5 ml 2 X YT, 1 % glucose, 100 µg/ml Ampicillin overnight at 30 °C. IPTG was added at 1 mM for a 3 hr induction period. Soluble scFv-CBD fusion proteins
 10 were isolated from each clone by sonication of induced cell pellets. In order to identify functional soluble 508(Fv) from non-functional ones, 250 ng/well β -amyloid peptide were covalently bound to epoxy-coated microtiter plates for 16 hr at 4 °C (Solomon, B., *et al.*, Proc. Natl. Acad. Sci. USA., 93:452-455,1996). The plates were washed with PBS/0.05 %
 15 - Tween 20 (PBST), and blocked with a mixture of 3 % bovine serum albumin and milk powder in PBS for 16 hr at 4 °C. The plates were then washed and incubated with the soluble scFv-CBD recovered from the clones for 1 hr at 37 °C. The bound antibody was detected with a rabbit anti CBD antiserum followed by HRP-conjugated goat anti rabbit

antibodies. Plates were developed with the peroxidase chromogenic substrate ABTS and the signal was recorded with an ELISA microtiter plate reader at 405 nm. Positive phage clones (pCC-508(Fv)) were propagated and their DNA was sequenced using an automated model 5 373A DNA sequencer (Applied Biosystems, USA).

Production of 508(Fv)-CBD fusion proteins in E. coli:

For high level expression in *E. coli*, wild type (wt) and mutated 508(Fv) derivatives were cloned into the pFEKCA3 vector as described (Berdichevsky Y *et al*, Protein Expr Purif., 17(2):249-59, 1999). This 10 vector utilizes the strong T7 promoter for expression, where the T7 RNA polymerase gene is carried as a lac repressor controlled-IPTG inducible gene in *E. coli* BL21 (DE3) (Studier, F.W., *et al.*, Methods Enzymol., 85, 60-89, 1990). Upon IPTG induction, 508(Fv)-CBD proteins accumulated as insoluble inclusion bodies. They were recovered by the cellulose- 15 assisted refolding method as previously described (Berdichevsky Y *et al*, Protein Expr Purif., 17(2):249-59, 1999). SDS polyacrylamide gel electrophoresis (SDS/PAGE) was used to separate proteins according to their molecular weight under denaturing conditions (Laemmli, U.K., Nature 227:263-270,1970).

Stability assay of the purified 508(Fv)-CBD protein:

The activity of purified 508(Fv)-CBD protein was checked before and after storage at 4 °C for 7 days. 250 ng/well β -amyloid peptide was covalently bound to epoxy-coated wells of microtiter plates for 16 hr at 4 °C (Solomon B. *et al.*, Proc. Natl. Acad. Sci. USA. 94, 4109-4112, 1997).

Wells were blocked with a mixture of 3 % bovine serum albumin and bovine hemoglobin in PBS for 2 hr at 37 °C, then washed and incubated with the 508(Fv)-CBD protein (0.5 μ g/ml or as otherwise specified) for 1 hr at 37 °C. Bound antibody fragments were detected by incubation with HRP-conjugated rabbit anti-mouse antibodies (BioMakor, Rehovot, Israel), diluted 1:5,000 and rabbit anti CBD diluted 1:10,000 in PBST for 1 hr at 37 °C. The bound antibody fragments were monitored as described above.

Construction of a phage library for the isolation the 508(Fv) β

AP binding mutants:

Splicing overlap extension (SOE) PCR technique (Lefenbrve, B., *et al.*, Biotechniques, 19:186-188, 1995) was used to replace V_L cysteine codon 96 of 508(Fv) with other codons. pCC-508(Fv) DNA was used as

template. In a first step, the template DNA was amplified with the following primers:

The antisense primer 508-mut-FOR: 5'-CCCCCTCCGAAC
GTSNATGGGTAACTcgatcgCTGATGGCAGTA-3' (SEQ ID NO:10)

5 inserts a *PvuI* restriction site (underlined), where S represents nucleotides C or G and N represents A, C, T or G. This primer was used for the replacement of cysteine codon 96 with phenylalanine (F), leucine (L), serine (S), tyrosine (Y) or tryptophan codons. The primer *SfiI* 5'BACK: 5'-ATCTATGCggcccagccggccATG-3' (SEQ ID NO:11) inserts an *SfiI*
10 site at the 5' end of the scFv. The resulting PCR product (*SfiI*-508mut) corresponds to the 5' half of 508(Fv)-CBD. In the second PCR step, the complete 508(Fv)-CBD was re-assembled by amplifying pCC-508(Fv) DNA with the *SfiI*-508mut PCR product from step 1 serving as the 5' end primer and CBD(BX): 5'-GTGGTGCTGAGTggatccta
15 TACTACACTGCCACCGGG-3' (SEQ ID NO:12) as the 3' end primer.

— The final PCR product (*SfiI*-508mut-BX) is a complete 508(Fv)-CBD cassette with replacements at V_L codon 96 and an engineered *PvuI* restriction site as a silent mutation for analysis. *SfiI*-508mut-BX DNA was digested with *SfiI*, *PvuI* and *NotI* and ligated in a three fragment

ligation with *Sfi*I and *Not*I linearized pCC-Gal6(Fv) DNA which is a phagemid vector used to display an anti *E. coli* β -galactosidase scFv (Berdichevsky Y *et al*, J Immunol Methods., 31;228(1-2):151-62, 1999).

The resulting ligated phagemid DNA was introduced into *E. coli* XL-1-
 5 Blue cells by electroporation. Cultures of *E. coli* were used to produce displaying phage by rescue with M13KO7 helper phage (Pharmacia Biotech, Uppsala, Sweden).

Affinity selection of β -amyloid binding 508mut-(Fv) displaying phage clones:

10 A sample containing rescued phage particles was subjected to one round of affinity selection (biopanning) and amplification. For the selection cycle, 0.5 μ g/ml biotinylated β -amyloid 1-16 amino acid peptide (β AP(1-16), acids 1-16 OF SEQ ID NO:3) in a total volume of 1 ml were used. The phages were pre-incubated with the biotinylated
 15 peptide for 2 hr at room temperature, and the reaction mixture was then layered on streptavidin-coated 30 mm polystyrene Petri dishes and incubated for 20 min at room temperature. Unbound phages were removed by extensive washing with PBST. The bound phages were
 — eluted with 0.3 ml of 0.1 M HCl titrated to pH 2.2 with glycine. The

eluate was neutralized with 80 μ l of 0.5 M Tris (HCl) pH 10, and used to infect *E. coli* XL-1-Blue cells. Individual bacterial colonies containing amplified phage particles were used as a template for colony PCR (Novagen Madison, USA) with primers *Sfi*15'Back and CBD(BX). The

5 PCR product of about the size of an intact scFv-CBD fragment (about 1250 bp) was digested with the restriction enzyme *Pvu*I and analyzed by agarose gel electrophoresis.

ScFv binding to biotinylated β AP(1-16):

Binding of scFv to β AP(1-16) was analyzed by ELISA. Coated

10 plates with 50 μ l of 1 μ g/ml streptavidin in 0.1 M NaHCO₃, pH 9.6, were washed three times with PBST and 50 μ l of 6 ng/ μ l biotinylated β AP(1-16) were then added to the wells and incubated for 30 min at 37 ° C. Wells were blocked with a mixture of 3 % bovine serum albumin and bovine hemoglobin in PBS for 2 hr at 37 °C, then washed and incubated

15 with the scFv (0.5 μ g/ml or as otherwise specified) for 1 hr at 37 °C. For inhibition experiments, peptides were pre-incubated with the antibody for 30 min at 37 °C before their addition to peptide coated wells. After washing, bound antibody fragments were detected as described above. β

AP specific binding phage clones were propagated and their DNA was isolated and sequenced as described above.

Cell culture and cytotoxicity assay:

Rat pheochromocytoma PC12 cells were cultured in DMEM
5 supplemented with 5 % horse serum, 10 % fetal calf serum, 2 mM L-
glutamine, and 100 units/ml penicillin/streptomycin and incubated at 37 °
C under 5 % CO₂. For the neurotoxicity assay, cultured PC12 cells were
seeded into a 96-well plate at a density of 10⁴ cells/100 µl/well in a
serum-free medium supplemented with 2 M of insulin. The effect on the
10 prevention of the neurotoxicity of βA was measured as follows: 0.12 mM
β-amyloid that was incubated for a week at 37 °C for the generation of
fibrils, and further incubated in the presence of 508F(Fv)-CBD or with
the unrelated Gal6(Fv)-CBD at a molar ratio of βAP to scFv of 15:1 or
30:1 for 24 hr. The βA/antibody mixture was added to the wells
15 containing PC12 cells. The plates were incubated at 37 °C for 2 days,
— after which cell viability was assessed by measuring cellular redox
activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium
bromide (MTT), as described (Sladowski, D. *et al.*, J. Immunol.
Methods., 157:203-207,1993). The plates were incubated overnight at 37

°C. MTT reduction was determined colorimetrically using an ELISA microtiter plate reader set at 550 nm.

Thioflavin T (ThT) fluorimetry:

Aggregation of β -amyloid peptide was measured by the Thioflavin
 5 T (ThT) binding assay, in which the fluorescence intensity reflects the
 degree of β -amyloid fibril formation. ThT characteristically stains
 amyloid-like deposits (Levine, H. III, Protein Sci., 2: 404-410,1993) and
 exhibits enhanced fluorescence emission at 485 nm upon excitation at
 435 nm when added to the suspension of aggregated β -sheet
 10 preparations. Aqueous solutions of 0.12 mM β AP in 0.1 M Tris (HCl)
 pH 7.1 were incubated at 37 °C for 1 week and further incubated in the
 presence of 508F(Fv)-CBD or with the unrelated Gal6(Fv)-CBD at a
 molar ratio of β AP to scFv of 15:1 or 30:1 for 24 hr. The fluorescence
 was measured after addition of 1 ml of ThT (2 μ M in 50 mM Glycine,
 15 pH 9) with an LSB-50 Perkin Elmer Ltd., UK, spectrofluorimeter.

Preparations of phage delivery system:

12 Balb/c-female mice were divided to four groups of 3 mice per
 group. One group was used as control. Following a single dose of 10^{11}
 phage particles (fd phage, taken from a 15-mer phage peptide library

which was provided by George P. Smith, University of Missouri, Columbia, MO) administered intranasally, mice were sacrificed in intervals of 1, 14 and 28 days in each group and their brains were taken for further analysis.

5 *Ability of phage carrying scFv to enter/remove β AP fragment from the brain:*

ScFv-508F fusion to filamentous minor coat gpIII were used in order to investigate the ability of β AP anti-aggregating scFv to be carries by a fillamentous phage display system directly into the CNS.

10 This scFv was prepared from anti-aggregation hybridoma 508 as described above and preserved its specific binding activity. Nine Balb/c mice divided into three groups were treated as follows: Mice of a first group were treated with 0.2 ml of 10^{-3} M biotin β A(1-16) alone. Mice of a second group were treated with a mix of 10^{10} phage carrying 508F-
15 scFv which were pre incubated with 0.2 ml of 10^{-3} M biotin β A(1-16) — for 1 hr. Mice of a third group were used as control. Following a single dose applied intranasally, mice were sacrificed in each group in intervals of 1, 14 and 28 days and their brains were taken for further analysis.

Preparations of tissue sections:

Immediately following decapitation, brains were removed and cut into two halves along the mid-sagittal sinus. Randomly, one half-brain was fixed by immersion in 4 % paraformaldehyde solution in 0.1 M phosphate buffer for two hours in 4 °C and then immersed for cold protection in 4.5 % sucrose in 0.1 M PBS over night. The sections were then moved to 30 % sucrose for 2 hr in 4 °C. Sections of coronal blocks containing the olfactory and hippocampus were put in OCT and cut with thick-nesses of 6 μ m with a cryostat at - 20 °C, and then taken up on glass slides. Slides were kept at -70 °C. These slides were used for phage detection using an immunofluorescence technique.

The other mid-sagittal half-brain was used for preparing paraffin tissue section for histology. The section were fixed in 4 % paraformaldehyde for 2 hr, then transferred to 10 % formalin saline for 2 days in room temperature, followed by embedding in paraffin, and cut with thick-nesses of 4 μ m on a microtome and then taken up on glass slides. The slides were kept at room temperature until used.

Detection of antigen in brain sections:

Immunofluorescence: Sections were blocked with 3 % bovine serum albumin in PBS for 30 min and then incubated with rabbit polyclonal serum anti fd (1:100) or Streptavidin coupled with PE (sigma) for 1 hr at 37 °C. Slides were then washed three times, 5 min each, in PBS, treated again with the blocking buffer for 5 minutes at room temperature, and then reacted with secondary r CyTM 3 donkey anti-rabbit IgG (for phage detection) at 1:400 (sigma) or with streptavidin coupled to PE, 1:50 dilution, for 1 hr at room temperature. Finally, the preparations were washed three times in PBS, observed using a fluorescence microscope at a final magnification of x 10, and recorded on film or using a Hamamatsu digital camera (C4742) and Metamorph (Universal Imaging; West Chester, PA) computer software.

Histology: Six-micrometer sections were stained with hematoxylin and eosin. The stained sections were examined and photographed at a final magnification of x 40. Finally, the preparations were washed three times in PBS, observed on a microscope, and recorded on film.

Immunization with f3-YYEFRH:

Immunizations were performed with a genetically engineered fd phage carrying the peptide YYEFRH (SEQ ID NO:7) fused to its minor coat gpIII. Doses of 10^{10} phages per injection were used to immunize at
5 14-day intervals, through intraperitoneal injections. Mice were injected the phages with or without Freund's complete adjuvant (Difco) for the first injection and Freund's incomplete adjuvant (Difco) for the second injection. Following 7 days of each injections, the mice were bled and their serum were tested by ELISA for antibody IgG reactivity for both
10 phage coat proteins and for β A.

Epitope libraries:

The 15-mer phage-peptide library used in this study was provided by George P. Smith (University of Missouri, Columbia, MO). The library consists of about 1.9×10^9 phage particles and comprises a random
15 peptide repertoire of 15 amino acid residues fused to coat glycoprotein VIII of the fd phage. Experiments with this library were carried out according to instructions of the provider (George P. Smith University of Missouri, Columbia, MO).

Biotinylation of antibodies:

For antibody biotinylation, 100 μg of each antibody in 0.1 M NaHCO_3 , pH 8.6, was incubated for 2 hr at room temperature with 5 μg of biotinamidocaproate N-hydroxysuccinimide ester (Sigma, B 2643) from a stock solution of 1 mg/ml in dimethylformamide and dialyzed at 4 °C against phosphate-buffered saline (PBS; 0.14M NaCl/0.01 M phosphate buffer, pH 7.4) overnight.

Isolation of phage presenting epitopes from peptide library:

A library sample containing 10^9 infectious phage particles was subjected to three rounds of selection (biopanning) and amplification. For each selection cycle a biotinylated monoclonal antibody (1 $\mu\text{g}/\mu\text{l}$) in a total volume of 25 μl was used. The phage clones were pre incubated with the biotinylated antibody overnight at 4 °C, and the reaction mixtures were then layered in 1 ml of PBS containing 0.5 % Tween 20 on streptavidin-coated 30 mm polystyrene Petri dishes and incubated for 20 min at room temperature. Unbound phages were removed by extensive washing (10 times for 10 min each) in PBS/0.05 % Tween 20. The bound phages were eluted with 0.3 ml of 0.1 M HCl titrated to pH 2.2 with glycine. The eluate was neutralized and used to infect *E. coli*

K91 cells. After three rounds of panning individual bacterial colonies containing amplified particles were grown on a microtiter plate and the selected phages were tested by ELISA for their ability to bind to the studied antibody, as described below.

5 *Antibody binding to isolated phage:*

Binding of antibodies to phage was analyzed by ELISA. Wells of microtiter plates (Maxisorb, Nunc) were coated with 50 μ l (at dilution of 1:1000 in 0.1 M NaHCO_3 , pH 8.6) of rabbit anti-phage serum and incubated overnight at 4 °C. The wells were blocked with a mixture of
10 3 % bovine serum albumin and hemoglobin at a ratio of 1:1 (in PBS) for 2 hr at 37 °C. Coated plates were washed three times with PBS/0.05 % Tween 20, and 50 μ l of enriched phage clones containing 10^{10} phage particles were added to the wells and incubated for 1 hr at 37 °C. After washing, the studied antibody was added (1 μ g/ml or as otherwise
15 specified) and allowed to bind to the coated plate overnight at 4 °C and the binding constant thereof was measured. Positive phage clones were propagated and their DNA were sequenced in the insert region at the Sequencing Unit of the Weizmann Institute of Science (Rehovot, Israel)
— by using Applied Biosystem Kit (United States, Applied Biosystem).

fd gpVIII phage display of β A(1-16):

Coat glycoprotein VIII of filamentous phage is presented in approximately 2700 copies on the phage coat. The following oligonucleotides were prepared: sense - 5'-agctccGATGCTGAATTCGG
 5 TGATAGCGGCTACGAAGTGCATCATCAGAAAcctgcag-3' (SEQ ID NO:13); and antisense - 5'-ggTTTCTGATGATGCACTTCGTAGC
 CGCTATCATGACGAAATTCAGCATCgg-3' (SEQ ID NO:14). These oligonucleotides were used to form a duplex (68-70 ° C, 10 minutes, followed by slow cool to room temperature) which encodes for amino
 10 acids 1-16 of human β AP and contain a silent mutation of a specific restriction site (*EcoRI*) which is useful for further analysis. The duplex was phosphorylated and ligated into *HindIII/PstI* linearized f88-4 phagemid, which is a vector used to display fusion peptides on gpVIII of filamentous phage. The resulting ligated phagemid DNA was introduced
 15 into *E. coli* K91K cells by transformation and transformants were plated onto 2 x YT Agar plates containing 10 μ g/ml tetracycline and 1 % glucose for overnight growth at 37 °C. Individual bacterial colonies containing phage particles were used to inoculate 2YT medium containing 10 μ g/ml tetracycline for overnight growth at 37 °C for

amplification. The DNA phagemid product obtained from each colony was analyzed by EcoRI. Positive clones were further amplified for antigen preparation.

Immunization with f88-EFRH:

5 Immunizations were performed with a genetically engineered fd phage carrying the peptide VHEPHEFRHVALNPV (SEQ ID NO:8) fused to its major coat glycoprotein VIII. Doses of 10^{10} phages per injection were used to immunize at 14-day intervals, through intraperitoneal injections. Mice were injected the phages with or without
10 Freund's complete adjuvant (Difco) for the first injection and Freund's incomplete adjuvant (Difco) for the second injection. Following 7 days of each injections, the mice were bled and their serum were tested by ELISA for antibody IgG reactivity for both phage coat proteins and for β A.

15 *Inhibition of antibody binding to β -amyloid peptide:*

— The inhibition of antibody binding to β AP(1-16) by various small peptides was performed using 250 ng/well biotinylated β -amyloid peptides (1-16) bound covalently to ELISA plates as previously described. The plates were washed with PBS/0.05% Tween 20 and

blocked with a mixture of 3 % bovine serum albumin and hemoglobin, ratio 1:1 (in PBS) for 2 hr at 37 °C. The peptides were pre incubated with 1:3000 dilution of serum after third immunization with f88-EFRH for 30 min at 37 °C before their addition to β AP-coated wells and were
5 left overnight at 4 °C therein. After washing, bound antibody was detected by incubation with HRP-conjugated rabbit anti-mouse immunoglobulin, as described above. The results were used to derive the IC_{50} , which is the half molar concentration of peptide that fully inhibits antibody binding. Peptides were synthesis by Applied Biosystems
10 Synergy Model 430A in the Unit for Chemical Services of The Weizmann Institute of Science by solid-phase using Fmoc chemistry.

Cell culture and cytotoxicity assay:

Rat pheochromocytoma PC12 cells were cultured in DMEM supplemented with 5 % horse serum, 10 % fetal calf serum, 2 mM L-
15 glutamine, and 100 units/ml penicillin/streptomycin and incubated at 37 °C under 5 % CO_2 . For the neurotoxicity assay, cultured PC12 cells were seeded into a 96-well plate at a density of 10^4 cells/100 μ l/well in a serum-free medium supplemented with 2 M of insulin. The effect on the
prevention of the neurotoxicity of β A was measured as follows: 0.12 mM

β -amyloid that was incubated for a week at 37 °C for the generation of fibrils, and further incubated in the presence of serum of EFRH-phage immunized mice and serum of a non-relevant phage immunized mice at dilutions of 5:1 and 10:1 for 24 hr. The β A antibody mixture was added
5 to the wells containing PC12 cells. The plates were incubated at 37 °C for 2 days, after which cell viability was assessed by measuring cellular redox activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), as described (Sladowski, D. *et al.*, J. Immunol. Methods., 157:203-207,1993). The plates were incubated overnight at 37
10 °C. MTT reduction was determined colorimetrically using an ELISA microtiter plate reader set at 550 nm.

EXPERIMENTAL RESULTS

Examples 1-6 below relate to the production of a single chain version of the anti aggregating monoclonal antibody. Examples 7-8
15 below relate to delivery of peptide or antibody displaying phage to the brain. Examples 9-14 below relate to the production of high titers of anti-aggregating polyclonal antibodies by direct immunization with beta amyloid antigens displayed on a phage, and to characterization of these
antibodies.

EXAMPLE 1

Generation of an IgM hybridoma 508

Immunization of a mouse with a 16 amino acid peptide of beta-amyloid (acids 1-16 of SEQ ID NO:3) conjugated to KLH (SEQ ID
5 NO:9) was carried out as described hereinabove. Repetitious immunization eventually produced a low but measurable antibody titer against beta-amyloid. Subsequent splenectomy of the immunized mouse facilitated preparation of IgM hybridoma 508 expressing scFvAb with specificity to beta-amyloid. RNA was subsequently extracted from this
10 hybridoma. The IgM 508 hybridoma showed specific activity to A β in preventing its toxic affects on PC12 cells (Anavi, S. 1998, M. Sc. thesis from the department of Molecular Microbiology and Biotechnology of the Tel-Aviv University, I).

EXAMPLE 2

15 *Cloning of the variable domains of the 508 IgM hybridoma as a scFv*

MAb 508 showed specific recognition of β -amyloid and prevented its toxic affects on PC12 cells (Anavi S., 1998, *ibid*). For cloning the 508 antibody as a scFv in a phage display vector, RNA was extracted from
10⁸ 508 hybridoma cells and was used as a source for antibody variable

region coding sequences. RT-PCR was used to amplify the variable domains that were cloned into the phage display vector pCC-Gal6(Fv), as described in Materials and Methods. When hybridoma derived antibodies are cloned as scFvs, some of the clones may contain aberrant sequences that are not functional. Therefore, to identify phagemid clones carrying functional β -amyloid binders from the generated clones, 10 individual clones were picked at random and soluble scFv-CBD fusion protein was produced thereby. Figure 2 shows a physical map of plasmid pCC-508 which was used to express the 508-scFv. The CBD domain serves as an immunological detection of soluble scFv protein or as a novel approach in refolding of soluble scFv protein inclusion bodies of overexpressed protein (Berdichevsky Y *et al*, Protein Expr Purif., 17(2):249-59, 1999). The plasmid used for 508-scFv over expression is shown in Figure 3. The soluble scFv-CBD from the selected clones was incubated in wells of an ELISA plate that has been coated with β -amyloid peptide. Of the analyzed clones, 50 % showed specific binding to β AP. Figures 1a-e demonstrate and illustrate the preparation of 508 scFv from IgM antibody. Figure 4 shows β AP binding by the scFv-CBD produced by a positive clone that was chosen for further analysis. PCR

analysis was used to characterize its DNA insert. It was found that the positive clone (designated pCC-508(Fv)) contained an intact DNA insert (Figure 5). DNA sequencing of pCC-508(Fv) confirmed that the clone expresses an intact scFv fragment (see, Figures 11a-b and SEQ ID NOs:5
5 and 6, for nucleic and amino acid sequences, respectively, modified as further described below).

EXAMPLE 3

Site directed mutagenesis of 508-(Fv) antibody

The DNA sequencing analysis of pCC508-(Fv) revealed the
10 unusual appearance of a cysteine residue at the position 96 of V_L CDR3 (Kabat, E. A. *et al.*, Sequences of proteins of immunological interest, 5th Ed., 1991). The deduced amino acid sequence of V_L CDR3 is: H⁸⁹QRSSYPCT⁹⁷ (SEQ ID NO:15). The presence of an unpaired
cysteine residue in a scFv may reduce its folding yield and also decrease
15 its stability in solution and its storage half life. Therefore, 508(Fv) was subcloned into an expression vector and produced in *E. coli* as described in Materials and Methods. Figure 6 summarizes the production process of 508(Fv)-CBD by the cellulose-assisted refolding method
(Berdichevsky Y *et al.*, Protein Expr Purif., 17(2):249-59, 1999).

Although 508(Fv)-CBD could be purified to near homogeneity (Figure 6 lane 7) by this method, it refolded relatively poorly and was unstable upon storage at 4 °C (Figure 7). It was assumed that substitution of the cysteine with a different residue may increase the production yield and stability of the soluble scFv without having an adverse affect on its affinity (Kirprianov, M.S. *et al.*, Protein Engineering, 10:445-453, 1997).

For the replacement of the 508 V_L cysteine 96 codon SOE PCR was used, which enabled the replacement of Cys 96 with phenylalanine, leucine, serine, tyrosine or tryptophan codons. In addition, the PCR scheme employed permits the persistence of the cysteine residue at that position. 508(Fv) mutants were cloned into the pCC-Gal6(Fv) phage vector, resulting in the generation of a micro library (having 6 potential variant). The replacing residues chosen are generally acceptable at that CDR3 position, as they are found in various antibodies in the Kabat database (Kabat, E. A. *et al.*, Sequences of proteins of immunological interest, 5th Ed., 1991). However, different replacements may vary in their effect on β AP binding. To test which replacement maintains β AP binding, a single cycle of affinity selection was performed on the

508(Fv)-Mut micro phage library using biotinylated β AP(1-16) as a capturing antigen. PCR amplification and restriction analysis was used to monitor the enrichment of library clones after the affinity selection cycle. When the 508Mut-(Fv)-CBD DNA is digested with *PvuI*, a typical

5 restriction pattern is obtained upon agarose-gel electrophoresis and ethidium-bromide staining. The lower 750 and 500 bp fragments represent the 508Mut-(FV)-CBD DNA, while an intact 1250 bp fragment represent scFv-CBD from the pCC-Gal6(Fv) DNA which was used as a vector. It was found that before affinity selection the library was heavily

10 contaminated with the pCC-Gal(Fv) vector DNA. This is evident from the fact that the DNA of 18/19 randomly picked library clones was not cleaved at the *PvuI* site engineered adjacent to 508 V_L position 96. Only one of the 19 analyzed clones showed the expected restriction

pattern associated with a mutation (Figure 8a). However, after affinity

15 selection, 5/11 randomly selected clones showed the expected restriction pattern (Figure 8b). This indicates an enrichment factor of about 10 fold, which demonstrates the ability of 508 scFv mutants to bind the β AP(1-16) epitope. The DNA sequences of the 5 mutants were determined and

are shown in Table 1 below. Suitable replacements of 508 L_L cysteine
96 codon were found to be phenylalanine, serine or tyrosine.

TABLE 1

Lane (Figure 8b)	Amino Sequence	Acid	SEQ ID NO:
4	89HQRSSYP-C ⁹⁶ -T		16
5	89HQRSSYP-F ⁹⁶ -T		17
6	89HQRSSYP-Y ⁹⁶ -T		18
8	89HQRSSYP-F ⁹⁶ -T		19
11	89HQRSSYP-S ⁹⁶ -T		20

EXAMPLE 4

The recognition of β AP(1-16) by scFv 508 mutants.

For further examination of mutated 508 scFv derivatives, the
mutated genes were subcloned into an expression vector and
overexpressed in *E. coli*, as described for the wild type protein above.

- 10 The interactions of the various mutated 508-(Fv) proteins (Table 1) with
 β AP(1-16) were tested in an ELISA assay. Figures 9a-b show that while
the wild type 508-(Fv)-CBD binds at a half maximum binding (HMB) of
 10^{-5} M, all the mutants showed improved binding to β AP: the HMB of
— C96S and C96Y is 5×10^{-6} M and the HMB of C96F is 10^{-7} M. For
15 further examination the 508-scFv mutant that carries the C96F mutation
(508F(Fv)) was chosen, which show the higher affinity and shelf stability
(Figures 9a-b). The specificity of β AP(1-16) binding by 508F(Fv) was

tested in a competitive ELISA. As is shown in Figure 10, binding of the purified 508F(Fv)-CBD to β AP was inhibited by soluble β AP(1-16) peptide serving as the competitor in the liquid phase in a dose-dependent manner. Inhibition of 50 % binding was obtained at about 1 μ M competitor. Binding was not affected by an irrelevant peptide (WVLD, SEQ ID NO:4).

EXAMPLE 5

Prevention of the β -amyloid neurotoxic effect by 508F(Fv).

In order to find out whether 508F(Fv) exhibits a protective effect similar to the parental IgM antibody in preventing β A mediated neurotoxicity toward cultured cells, an *in vitro* test was applied using rat phenochromocytoma PC12 as described (Solomon B. *et al.*, Proc. Natl. Acad. Sci. USA., 94: 4109-4112,1997). Viability of the cells exposed to β A with or without antibody was measured. As shown in Figure 12, 508F(Fv) prevented the neurotoxicity of β A (90 % cell viability) at a molar ratio β AP:scFv of 15:1, while the unrelated scFv showed no effect. Purified CBD or the scFv alone had no affect on the cells.

EXAMPLE 6

Disaggregation of β -amyloid fibril by 508F(Fv)

To examine the effect of 508F(Fv) on disruption of the β A fibril (the toxic form of β AP) the ThT reagent that binds specifically to fibrillar structures (Levine, H. III, Protein Sci., 2: 404-410,1993) was used. The interference with the already formed β A fibril was measured at the same molar ratio of β AP:scFv as in the β A neurotoxic assay and was quantitated by ThT fluorimetry. Figure 13 shows that 508F(Fv) incubated with pre-formed β A fibrils disrupted the fibril structure indicating extensive deterioration of fibril morphology, as is evidenced by a substantial (62 %) decrease in ThT fluorescence.

EXAMPLE 7

Ability of filamentous phage to enter the CNS via olfactory track

Female Balb/c mice were treated with phage vector f88-EFRH via intranasal administration. The propose of this experiment was to check the ability of filamentous phage to reach the hippocampous region via olfactory tract. Since the phage is not carrying any specific molecule for targeting neuron cells, it should be vanished without causing any harm after several day following the administration. In order to investigate the

appearance of phage in the olfactory bulb and the hippocampous region double labeling of antibodies was used as follows: Rabbit polyclonal antibody anti filamentous phage and mouse monoclonal antibody against EFRH epitope fused to glycoprotein VIII of the phage surface. One day following a single intranasal administration of 10^{11} phages animals showed such phages in their olfactory bulb and hypcampous (Figures 14a-d). Seven days after the administration phages were detected in the olfactory bulb of only one mouse of the three tested, whereas no phages were revealed in the hypcampous. No evidence of phages was detectable 28 days following administration (Figures 15a-d). As shown in Figures 16a-d, no evidence of change in the neuron population of the brain of treated mice was evident.

EXAMPLE 8

Filamentous phage are suitable vehicle for carrying active antibody

fragment to the CNS

To check whether a filamentous phage can carry an antibody to the CNS via the olfactory track and still preserve the activity against β -amyloid a filamentous phage displaying 508F was incubated with 10^{-3} M biotinlated β AP(1-16), in order to form antibody antigen

immunocomplex. Balb/c mice were divided into two groups and were administered intranasally with two different antigens: 508F- β AP(1-16) immunocomplex and for comparison biotinlated β AP(1-16) alone. Following a single dose the mice were sacrificed and brain sections
 5 thereof prepared and reacted with streptavidin coupled to a fluorescent agent. Fluorescence was detected only in brain sections of mice that were administered with 508F- β AP(1-16), but not, or to a much lesser extent, in brain sections of mice that were administered with β AP(1-16) alone (Figures 17a-d). No histological findings characterized treated mice
 10 (Figures 18a-d).

It is therefore assumed that the phage act as an inert vehicle of antibody to the brain, carrying the β AP(1-16) molecule into the brain.

EXAMPLE 9

Raising anti-aggregating β AP antibody through immunization of mice

15

with f3-EFRH phage

— The anti-aggregating epitope within β AP (EFRH, SEQ ID NO:1) map to positions 3-6 of the amino acid sequence of β AP. In order to generate specific immune response against β AP, mice were immunized with genetically engineered fd phage carrying the peptide YYEFRH

(SEQ ID NO:7) fused to its minor coat gpIII according to the immunization schedule shown in Figure 19. Doses of 10^{10} phage particles per injection were used to immunize, at 14-day intervals, through intraperitoneal injection. Following 7 days of each injection, mice were bled and their sera tested by ELISA for IgG antibody reactivity against wild type phage (not bearing the peptide YYEFRH on its surface) and against β AP (Figures 20a-b). This route of administration a very high response against β AP (1:750) following the third injection. Furthermore, it was found that injection through phage carrying epitope is long lasting (Figure 21), it is none toxic and may be given without adjuvant. The phage vector is found to be an immunogenic tool to raise a high affinity immune response within 14 days from the first injection. The immune response against the peptide YYEFRH (SEQ ID NO:7) is low compared to the immune response against the entire phage and could be explained by the low copy number of the fusion gpIII on the phage envelope. Therefore, for further analysis phages displaying the epitope through glycoprotein VIII were employed.

EXAMPLE 10

Isolation of f88-EFRH peptide-displayed phage by an anti-aggregating mAb

To identify a disaggregating EFRH peptide epitope a phage-
 5 epitope library was screened with biotinylated antibody. After three
 cycles of panning and phage amplification, 90 individually isolated
 bacterial colonies were grown in microtiter plates and their phages were
 assayed for antibody binding. ELISA analysis revealed that of the phage-
 clones which were selected followed by three biopanning cycles, most
 10 (above 80%) bound specifically to anti-aggregating mAb, respectively.
 DNA from 6 positive clones was sequenced (Table 2). The sequence
 EFRH (SEQ ID NO:1) appeared in 4 clones, one additional clone had the
 sequence EPRH (SEQ ID NO:1), with only one residue replacement of
 proline with phenylalanine. In one additional clone, the inserted peptide
 15 bears the sequence of the three residues FRH (acids 2-4 of SEQ ID
 — NO:1), lacking the glutamate residue.

TABLE 2

Amino acid Sequence (name)	SEQ ID NO:	No. of Phages
VHEPHEFRHVALNPV (C3-II)	8	2
DTEFRHSSNNFSAVR (C7-II)	21	1
STEFRHQTTPLHPNS (C11-I)	22	1
KEPRHHIQHHERVIR (F8-II)	23	1
SAADFRHGSPPI SAF (D3-I)	24	1

Binding of anti-aggregating mAb to the EFRH-bearing phage was concentration dependent; half-maximal binding was obtained at an antibody concentration of 100 ng/ml, corresponding to 10^{-9} M (Figure 4) which resembles the level of binding of these antibodies to the whole peptide. One specific f88-EFRH phage (termed C3-II, table 2) showed
 5 higher level of avidity as is compared to the others (Figure 22). It may be due to higher level of EFRH epitope exposure on its surface. Binding tests of f88-EFRH or f3-EFRH with the same concentration of anti-aggregating antibody (1 μ /ml) demonstrated a higher number of EFRH
 10 epitope copies per phage which may lead to higher serum titer via phage immune response (Figure 23).

EXAMPLE 11

Raising anti-aggregating β AP antibody through immunization of mice with f88-phage

15 In order to generate the same specific immune response against β AP, mice were immunized with genetically engineered fd phage carrying the peptide VHEPHEFRHVALNPV (SEQ ID NO:8) fused to its major coat gpVIII. This phage was selected from a 15-mer phage peptide
 library by an anti aggregating β AP antibody and is presenting the mAb

epitope (underlined) within β AP. This phage was used to immunize mice as described. Following 7 days of each injection with 10^{10} phage particles (without adjuvant) the mice were bled and their sera tested by ELISA for IgG antibody reactivity against wild type phage and against β AP. The results are summarized in Figures 24a-b. All animals showed a measurable response of IgG antibody against the wild type phage, and titers increased with the second and the third injection. This route also gave the highest titer measurable responses against β AP (1:50,000) after the third injection (Figure 24b).

EXAMPLE 12

Inhibition of antibody serum binding to β -amyloid peptide

The interaction of mouse serum immunized by phage f88-EFRH with β AP was further assayed by competitive inhibition experiments. Figure 25 shows inhibition of mice serum antibody with synthesized peptides derived from β AP, each of which includes the sequence EFRH) such as: DAEFRH (positions 1-6, SEQ ID NO:3), DAEFRHD (positions 1-7, SEQ ID NO:3), DAEFRHDSG (positions 1-9, SEQ ID NO:3), and β AP itself, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (positions 1-40, SEQ ID NO:3).

Figure 25 shows that all of the synthetic peptides which bear the motive EFRH (SEQ ID NO:1) similarly inhibited binding of mouse serum antibody to the β AP with IC₅₀ values of about 5×10^{-6} M. These data indicate that the epitope of mouse serum antibody within the β AP molecule is composed of four amino acid residues corresponding to positions 3-6 in the β AP which was found to act as a regulatory site controlling both the solubilization and the disaggregation process of the β A molecule.

EXAMPLE 13

10 *Prevention of the β -amyloid neurotoxic effect by serum antibody raised against EFRH-phage*

In order to find out whether serum of f88-EFRH immunized mice exhibits a protective effect similar to the parental IgG antibody in preventing β A mediated neurotoxicity toward cultured cells, the *in vitro* test using rat pheochromocytoma PC12 was applied as described 15 (Solomon B. *et al.*, Proc. Natl. Acad. Sci. USA., 94: 4109-4112, 1997). Viability of the cells exposed to β A with or without antibody was measured. As shown in Figure 26, diluted serum of 1:5 prevented the

neurotoxicity of β A (80 % cell viability), while the unrelated serum showed no effect.

EXAMPLE 14

Disaggregation of β -amyloid fibril by serum of EFRH immunized mice

5 To examine the effect of serum of f88-EFRH immunized mice on disruption of the β A fibril (the toxic form of β AP) the ThT reagent that binds specifically to fibrillar structures (Levine, H. III, Protein Sci., 2: 404-410,1993) was used. β AP samples were incubated for a week at 37 ° C and then were exposed to different dilutions mouse serum antibody.

10 Fibril formation was quantitated by the ThT fluorometry binding assay. Figure 27 shows that mouse serum, at dilution of 1:5 and 1:20, disrupted the fibril structure of β A with extensive deterioration of fibril morphology, as indicated by a substantial 75 % (1:5 dilution) and 50 % (1:20 dilution) decrease in ThT fluorescence. The unrelated serum used

15 as control (serum from non-immunized mouse), did not significantly inhibit fibril formation as is compared to the immunized serum. This result strongly emphasizes the ability of the EFRH epitope displayed by a filamentous phage vector to evoke an immune response resulting in anti-aggregation antibody.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications
5 and variations that fall within the spirit and broad scope of the appended claims. All publications cited herein are incorporated by reference in their entirety.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANTS: Beka Solomon et al.
 - (ii) TITLE OF INVENTION: IMMUNIZATION AGAINST
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 - (iii) NUMBER OF SEQUENCES: 24
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Mark M. Friedman c/o Anthony Castorina
 - (B) STREET: 20001 Jefferson Davis Highway, Suite 207
 - (C) CITY: Arlington
 - (D) STATE: Virginia
 - (E) COUNTRY: United States of America
 - (F) ZIP: 22202
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 1.44 megabyte, 3.5" microdisk
 - (B) COMPUTER: Twinhead* Slimnote-890TX
 - (C) OPERATING SYSTEM: MS DOS version 6.2,
Windows version 3.11
 - (D) SOFTWARE: Word for Windows version 2.0
converted to an ASCII file
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 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Friedmam, Mark M.
 - (B) REGISTRATION NUMBER: 33,883
 - (C) REFERENCE/DOCKET NUMBER: 27/153
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 972-3-5625553
 - (B) TELEFAX: 972-3-5625554
 - (C) TELEX:
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
Glu Phe Arg His
- (2) INFORMATION FOR SEQ ID NO:2:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln
5 10 15
Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
20 25 30
Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
35 40

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Trp Val Leu Asp

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 717
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGGTCAAAC TGCAGGAGTC AGGGGCTGAG CTGGTGAGGC CTGGGGTCTC 50
AGTGAAGATT TCCTGCAAGG GTTCTGGCTA CACATTCACT GATTATGCTA 100
TGCACTGGGT GAAGCAGAGT CATGCAAAGA GTCTAGAGTG GATTGGAGTT 150
ATTAGTACTT ACTATGGTGA TGCTAGCTAC AACCAGAAGT TCAAGGGCAA 200
GGCCACAATG ACTGTAGACA AATCCTCCAG CACAGCCTAT ATGGAAGTCTG 250
CCAGACTGAC ATCTGAGGAT TCTGCCATCT ATTACTGTGC AAGAGGGGCT 300
ACTATGTCTT ACTTTGACTA CTGGGGCCAA GTGACCACGG TCACCGTCTC 350
CTCAGGTGGA GCGGGTTCAG GCGGAGTTGG CTCTGGCGGT GCGGATCGG 400
ACATCGAGCT CACTCAGTCT CCAGCAATCA TGTCTGCATC TCAGGGGAG 450
AAGGTCACCA TGACCTGCAG TGCCAGCTCA AGTATAAGTT ACATGCACTG 500
GTATCAGCAG AAGCCAGGCA CCTCCCCCAA AAGATCGATT TATGACACAT 550
CCAAACTGGC TTCTGGAGTC CCTGCTCGCT TCAGTGGCAG TGGGTCTGGG 600
ACCTCTTATT CTCTCACAAT CAGCAGCATG GAGGCTGAAG ATGCTGCCAC 650
TTATTAAGTC CATCAGCGGA GTAGTACCC ATTCACGTTT GGAGGGGGGG 700
CCAAGCTGGA AATAAAA 717

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 244
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gln Val Lys Leu Gln Glu Ser Gly Ala Glu Leu Val Arg Pro Gly
5 10 15
Val Ser Val Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe Thr
20 25 30
Asp Tyr Ala Met His Trp Val Lys Gln Ser His Ala Lys Ser Leu
35 40 45
Glu Trp Ile Gly Val Ile Ser Thr Tyr Tyr Gly Asp Ala Ser Tyr
50 55 60
Asn Gln Lys Phe Lys Gly Lys Ala Thr Met Thr Val Asp Lys Ser
65 70 75
Ser Ser Thr Ala Tyr Met Glu Leu Ala Arg Leu Thr Ser Glu Asp
80 85 90
Ser Ala Ile Tyr Tyr Cys Ala Arg Gly Ala Thr Met Ser Tyr Phe
95 100 105
Asp Tyr Trp Gly Gln Val Thr Thr Val Thr Val Ser Ser Gly Gly
110 115 120
Gly Gly Ser Gly Gly Val Gly Ser Gly Gly Gly Ser Asp Ile

Glu	Leu	Thr	Gln	125	Ser	Pro	Ala	Ile	Met	130	Ser	Ala	Ser	Pro	Gly	135
Lys	Val	Thr	Met	140	Thr	Cys	Ser	Ala	Ser	145	Ser	Ser	Ile	Ser	Tyr	150
His	Trp	Tyr	Gln	155	Gln	Lys	Pro	Gly	Thr	160	Ser	Pro	Lys	Arg	Trp	170
Tyr	Asp	Thr	Ser	175	Lys	Leu	Ala	Ser	Gly	180	Val	Pro	Ala	Arg	Phe	185
Gly	Ser	Gly	Ser	190	Gly	Thr	Ser	Tyr	Ser	195	Leu	Thr	Ile	Ser	Ser	200
Glu	Ala	Glu	Asp	205	Ala	Ala	Thr	Tyr	Tyr	210	Cys	His	Gln	Arg	Ser	215
Tyr	Pro	Phe	Thr	220	Gly	Gly	Gly	Ala	Lys	225	Leu	Glu	Ile	Lys	Ser	230
				235						240						

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 -
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Tyr Glu Phe Arg His

5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val His Glu Pro His Glu Phe Arg His Val Ala Leu Asn Pro Val

5

10

15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Leu His

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCCCTCCG AACGTSNATG GGTAACTCGA TCGCTGATGG CAGTA 45

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCTATGCCG CCCAGCCGGC CATG 24

(2) INFORMATION FOR SEQ ID NO:12:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
 GTGGTGCTGA GTGGATCCTA TACTACACTG CCACCGGG 38

(2) INFORMATION FOR SEQ ID NO:13:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 58
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
 AGCTCCGATG CTGAATTCGG TGATAGCGGC TACGAAGTGC ATCATCAGAA ACCTGCAG 58

(2) INFORMATION FOR SEQ ID NO:14:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 52
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
 GGTTTCTGAT GATGCACTTC GTAGCCGCTA TCATGACGAA ATTCAGCATC GG 52

(2) INFORMATION FOR SEQ ID NO:15:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
 His Gln Arg Ser Ser Tyr Pro Cys Thr
 5

(2) INFORMATION FOR SEQ ID NO:16:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
 His Gln Arg Ser Ser Tyr Pro Cys Thr
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(2) INFORMATION FOR SEQ ID NO:17:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
 His Gln Arg Ser Ser Tyr Pro Phe Thr
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(2) INFORMATION FOR SEQ ID NO:18:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Gln Arg Ser Ser Tyr Pro Tyr Thr

5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Gln Arg Ser Ser Tyr Pro Phe Thr

5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

His Gln Arg Ser Ser Tyr Pro Ser Thr

5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Thr Glu Phe Arg His Ser Ser Asn Asn Phe Ser Ala Val Arg

5

10

15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ser Thr Glu Phe Arg His Gln Thr Thr Pro Leu His Pro Asn Ser

5

10

15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Glu Pro Arg His His Ile Gln His His Glu Arg Val Ile Arg

5

10

15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Ala Ala Asp Phe Arg His Gly Ser Pro Pro Ile Ser Ala Phe
5 10 15

[illegible]

WHAT IS CLAIMED IS:

1. A method of treating a plaque forming disease comprising the steps of:

- (a) displaying a polypeptide on a display vehicle, said polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said at least one epitope being capable of eliciting antibodies capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein;
- (b) introducing said display vehicle into a body of a recipient so as to elicit said antibodies capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.

2. The method of claim 1, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease,

SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

3. The method of claim 1, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

4. The method of claim 1, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

5. The method of claim 1, wherein said display vehicle is a virus.

6. The method of claim 5, wherein said virus is a bacteriophage.

7. The method of claim 6, wherein said bacteriophage is a filamentous bacteriophage.

8. The method of claim 6, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.
9. The method of claim 6, wherein said bacteriophage is capable of propagation in *Escherichia coli*.
10. The method of claim 6, wherein said bacteriophage is fd.
11. The method of claim 1, wherein said display vehicle is an *in vivo* non-propagateable particle.
12. The method of claim 1, wherein said display vehicle is selected such that less than 30 days following an introduction of a triple dose of 10^{10} units thereof to the recipient, a titer of said antibodies is above 1:50,000, as is determined by ELISA.
13. An agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide, said polypeptide representing at least one epitope of an aggregating protein associated with plaque

formation in said plaque forming disease, said at least one epitope being capable of eliciting antibodies capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.

14. The agent of claim 13, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

15. The agent of claim 13, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

16. The method of claim 13, wherein said display vehicle is — selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

17. The agent of claim 13, wherein said display vehicle is a virus.

18. The agent of claim 17, wherein said virus is a bacteriophage.

19. The agent of claim 17, wherein said bacteriophage is a filamentous bacteriophage.

20. The agent of claim 17, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

21. The agent of claim 17, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

22. The agent of claim 17, wherein said bacteriophage is M13.

23. The agent of claim 13, wherein said display vehicle is an *in vivo* non-propagateable particle.

24. The agent of claim 13, wherein said display vehicle is selected such that less than 30 days following an introduction of a triple dose of 10^{10} units thereof to the recipient, a titer of said antibodies is above 1:50,000, as is determined by ELISA.

25. A pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide, said polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said at least one epitope being capable of eliciting an effective amount of antibodies capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein, and a pharmaceutically acceptable carrier.

26. The pharmaceutical composition of claim 25, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary

Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

27. The pharmaceutical composition of claim 25, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

28. The pharmaceutical composition of claim 25, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

29. The pharmaceutical composition of claim 25, wherein said display vehicle is a virus.

30. The pharmaceutical composition of claim 29, wherein said virus is a bacteriophage.

31. The pharmaceutical composition of claim 30, wherein said bacteriophage is a filamentous bacteriophage.

32. The pharmaceutical composition of claim 30, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

33. The pharmaceutical composition of claim 30, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

34. The pharmaceutical composition of claim 30, wherein said bacteriophage is fd.

35. The pharmaceutical composition of claim 25, wherein said display vehicle is an *in vivo* non-propagateable particle.

36. The pharmaceutical composition of claim 25, wherein said display vehicle is selected such that less than 30 days following an

introduction of a triple dose of 10^{10} units thereof to the recipient, a titer of said antibodies is above 1:50,000, as is determined by ELISA.

37. A method of preparing a display vehicle for treating a plaque forming disease, the method comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said at least one epitope being capable of eliciting antibodies capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein, such that when said display vehicle propagates said polypeptide is displayed by said display vehicle.

38. The method of claim 37, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

39. The method of claim 37, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

40. The method of claim 37, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

41. The method of claim 37, wherein said display vehicle is a virus.

42. The method of claim 41, wherein said virus is a bacteriophage.

43. The method of claim 42, wherein said bacteriophage is a filamentous bacteriophage.

44. The method of claim 42, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

45. The method of claim 42, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

46. The method of claim 42, wherein said bacteriophage is fd.

47. The method of claim 37, wherein said display vehicle is selected such that less than 30 days following an introduction of a triple dose of 10^{10} units thereof to the recipient, a titer of said antibodies is above 1:50,000, as is determined by ELISA.

48. A method of treating a plaque forming disease comprising the steps of:

- (a) displaying a polypeptide on a display vehicle, said polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said

aggregating protein and/or of preventing aggregation of said aggregating protein; and

- (b) introducing said display vehicle into a body of a recipient so as to disaggregate said aggregating protein.

49. The method of claim 48, wherein introducing said display vehicle into the body of the recipient so as to disaggregate said aggregating protein is by applying said display vehicle to an olfactory system of the recipient.

50. The method of claim 48, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

51. The method of claim 48, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

52. The method of claim 48, wherein said display vehicle is a virus.

53. The method of claim 48, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

54. The method of claim 52, wherein said virus is a bacteriophage.

55. The method of claim 53, wherein said bacteriophage is a filamentous bacteriophage.

56. The method of claim 53, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

57. The method of claim 53, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

58. The method of claim 53, wherein said bacteriophage is fd.
59. The method of claim 48, wherein said display vehicle is an *in vivo* non-propagateable particle.
60. An agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.
61. The agent of claim 60, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.
-
-

62. The agent of claim 60, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

63. The agent of claim 60, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

64. The agent of claim 60, wherein said display vehicle is a virus.

65. The agent of claim 64, wherein said virus is a bacteriophage.

66. The agent of claim 65, wherein said bacteriophage is a filamentous bacteriophage.

67. The agent of claim 65, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

68. The agent of claim 65, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

69. The agent of claim 65, wherein said bacteriophage is fd.

70. The agent of claim 60, wherein said display vehicle is an *in vivo* non-propagateable particle.

71. An pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein, the pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

72. The pharmaceutical composition of claim 71, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

73. The pharmaceutical composition of claim 71, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

74. The method of claim 71, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

75. The pharmaceutical composition of claim 71, wherein said display vehicle is a virus.

76. The pharmaceutical composition of claim 75, wherein said virus is a bacteriophage.

77. The pharmaceutical composition of claim 76, wherein said bacteriophage is a filamentous bacteriophage.

78. The pharmaceutical composition of claim 76, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

79. The pharmaceutical composition of claim 76, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

80. The pharmaceutical composition of claim 76, wherein said bacteriophage is fd.

81. The pharmaceutical composition of claim 71, wherein said display vehicle is an *in vivo* non-propagateable particle.

82. A method of preparing a display vehicle for treating a plaque forming disease, the method comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.

83. The method of claim 82, wherein the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma and a spongiform encephalopathy.

84. The method of claim 82, wherein said aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cysantoin C, IgG kappa light chain and prion protein.

85. The method of claim 82, wherein said display vehicle is a virus.

86. The method of claim 82, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

87. The method of claim 85, wherein said virus is a bacteriophage.

88. The method of claim 86, wherein said bacteriophage is a filamentous bacteriophage.

89. The method of claim 86, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

90. The method of claim 86, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

91. The method of claim 86, wherein said bacteriophage is fd.
92. The method of claim 82, wherein said display vehicle is an *in vivo* non-propagateable particle.
93. The method of claim 48, wherein said step of introducing said display vehicle into said body of said recipient so as to disaggregate said aggregating protein is effected through an olfactory system of said recipient.
94. A method of introducing a display vehicle lacking an engineered targeting moiety into a brain of a recipient, the method comprising the step of administering said display vehicle intranasally to the recipient.
95. The method of claim 94, wherein said display vehicle is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, an RNA virus, a bacteria and a prion.

96. The method of claim 94, wherein said display vehicle is a virus.

97. The method of claim 96, wherein said virus is a bacteriophage.

98. The method of claim 97, wherein said bacteriophage is a filamentous bacteriophage.

99. The method of claim 97, wherein said bacteriophage is capable of propagation in bacterial flora in said recipient.

100. The method of claim 97, wherein said bacteriophage is capable of propagation in *Escherichia coli*.

— 101. The method of claim 97, wherein said bacteriophage is fd.

102. The method of claim 94, wherein said display vehicle is an ~~in vivo non-propagateable particle.~~

103. The method of claim 94, wherein the display vehicle displays at least an immunological portion of an antibody.

104. The method of claim 103, wherein said immunological portion of an antibody serves for binding at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.

ABSTRACT OF THE DISCLOSURE

A strategy for immunizing against amyloid plaques using display technology. The strategy includes methods, agents, and pharmaceutical compositions for vaccination against plaque forming diseases (e.g. Alzheimer's disease) which rely upon presentation of an antigen or epitope on a display vehicle. The strategy further includes methods, agents, and pharmaceutical compositions for vaccination against plaque forming diseases (e.g. Alzheimer's disease) which rely upon presentation of an antibody, or an active portion thereof, on a display vehicle. Whether antigens or antibodies are employed, desegregation of plaques results from the immunization.

Attorney Docket: 27/153
page 1 of 2

Combined Declaration For Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled IMMUNIZATION AGAINST AMYLOID PLAQUES USING DISPLAY TECHNOLOGY, the specification of which (check one) ☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____ and was amended on _____. I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

NONE

(number) (Country) (Day, Month, Year Filed)

(number) (Country) (Day, Month, Year Filed)

(number) (Country) (Day, Month, Year Filed)

Priority Claimed

☐ ☐

Yes No

☐ ☐

Yes No

☐ ☐

Yes No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

60/152,417

(Application Serial No.)

3 SEP 1999

(Filing Date)

PENDING

Status

(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

Status

(patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Mark M. Friedman Registration No. 33,883

Address all Correspondence to:

DR. MARK FRIEDMAN LTD.
c/o ANTHONY CASTORINA
2001 JEFFERSON DAVIS HIGHWAY
SUITE 207
ARLINGTON, VIRGINIA 22202

Direct all telephone calls & faxes to:
ROBERT SHEINBEIN
Phone (703) 415-1581
Fax (703) 415-4864

Attorney Docket. 27/153
page 2 of 2

Continuation of Combined Declaration For Patent Application and Power of Attorney

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application of any patent issued thereon.

*FULL NAME OF SOLE OR FIRST INVENTOR BEKA SOLOMON	INVENTOR'S SIGNATURE <i>Beka Solomon</i>	DATE Dec 26, 1999
RESIDENCE 120 HANASSI, HERZLIA PITUACH, ISRAEL	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS 120 HANASSI, HERZLIA PITUACH, ISRAEL		

*FULL NAME OF SECOND INVENTOR DAN FRENKEL	INVENTOR'S SIGNATURE <i>Dan Frenkel</i>	DATE Dec 26, 1999
RESIDENCE 32 GORODESKI, REHOVOT, ISRAEL	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS 32 GORODESKI, REHOVOT, ISRAEL		

*FULL NAME OF THIRD INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF FOURTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF FIFTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF SIXTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF SEVENTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

SMALL BUSINESS CONCERN - NEW APPLICATION

Attorney Docket No.: 27/153

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In RE Application of: BEKA SOLOMON ET AL.

Filed Concurrently Herewith

For: IMMUNIZATION AGAINST AMYLOID PLAQUES USING DISPLAY TECHNOLOGY

VERIFIED STATEMENT UNDER 37 CFR 1.27
CLAIMING STATUS AS A SMALL ENTITY

To The Commissioner of Patents and Trademarks:

I hereby declare that:

I am the owner of, or an official empowered to act on behalf of, the small business concern identified below:

Name of Concern: RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD

Address : 32 HAIM LEVANON, P. O. BOX 39296, 61392 TEL AVIV, ISRAEL

The small business concern identified above, together with its affiliates, employs fewer than 500 persons and qualifies as a small business concern as defined in 37 CFR 1.9(d) for purposes of paying reduced fees under 35 USC § 41(a) and § 41(b) to the Patent and Trademark Office with regard to the above-entitled invention described in the specification filed herewith

Rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above entitled invention.

If the rights held by the small business concern are not exclusive, each other party having rights to the invention is listed below, and no rights to the invention are held by any party who could not qualify as a small entity under 37 CFR 1.9(f), namely any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Full Name (Party 1) : NONE

Address : _____

Status : ☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Full Name (Party 2) : _____

Address : _____

Status : ☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

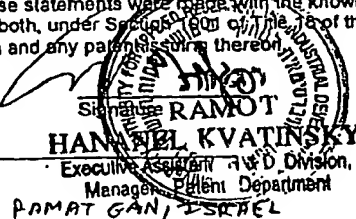
I acknowledge the duty under 37 CFR 1.28(b) to file, in this application, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the issue fee due after the date on which status as a small entity is no longer appropriate

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patents issuing thereon

Name of Person Signing

Capacity of Person Signing

Address of Person Signing :


Signature: RAMOT
HANNAH KVATINSKY
Executive Assistant, P.D. Division,
Manager, Patent Department
RAMAT GAN, ISRAEL

23-12-99

Date

RAMI FINKLER, Ph.D.
President/General Manager
RAHANA, ISRAEL

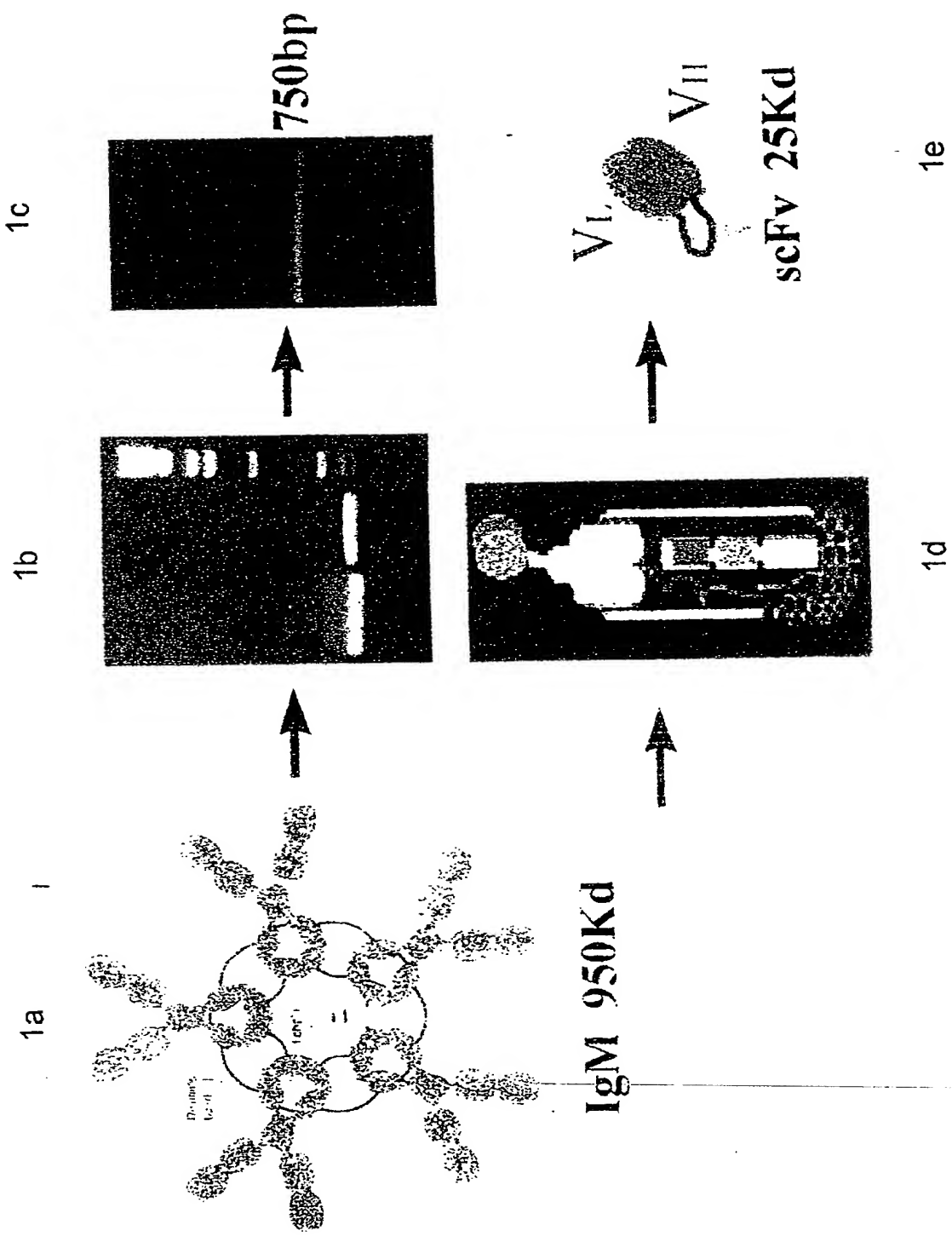


FIGURE 1

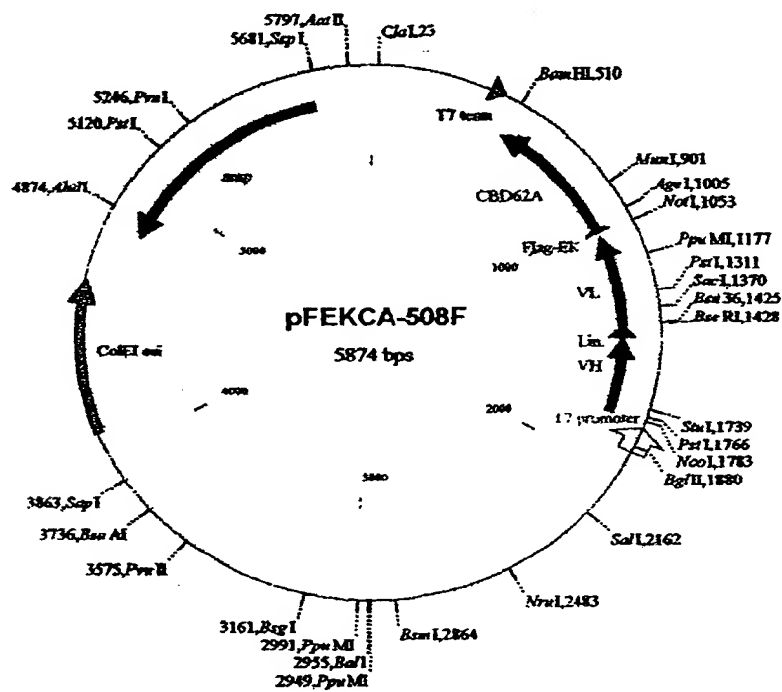


FIGURE 3

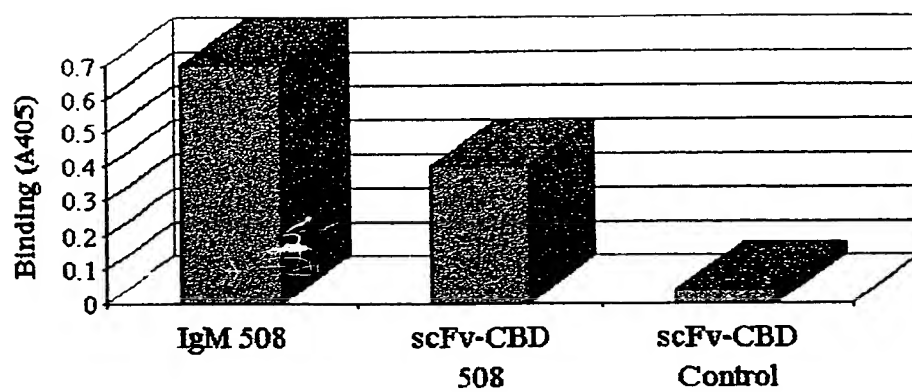


FIGURE 4

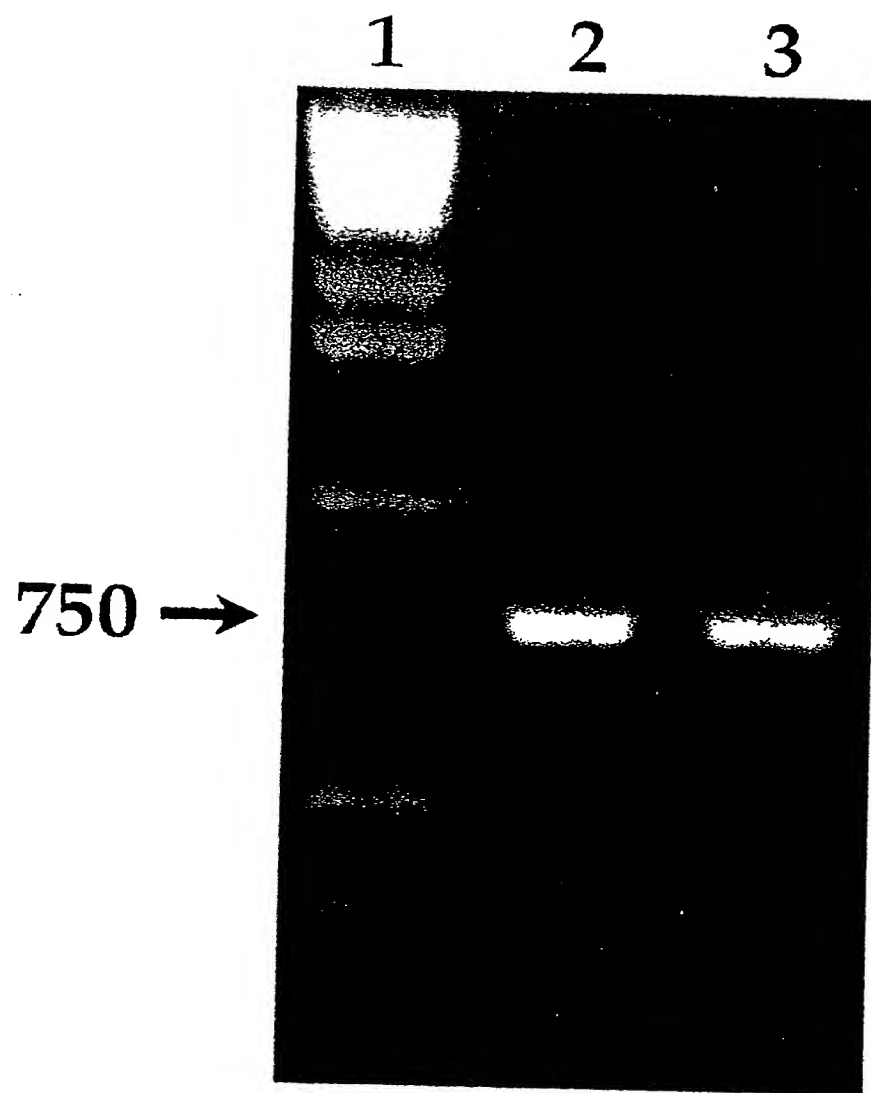


FIGURE 5

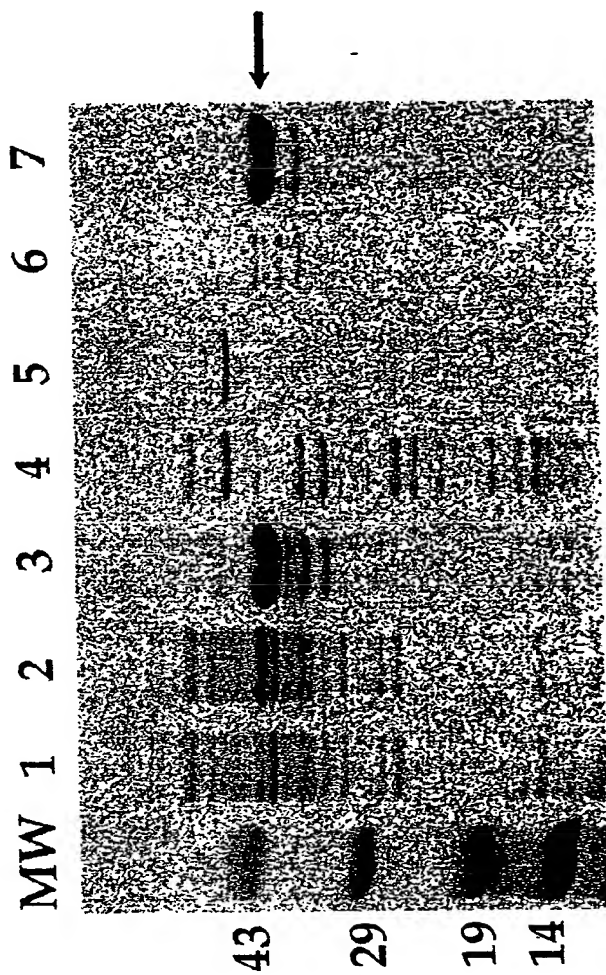


FIGURE 6

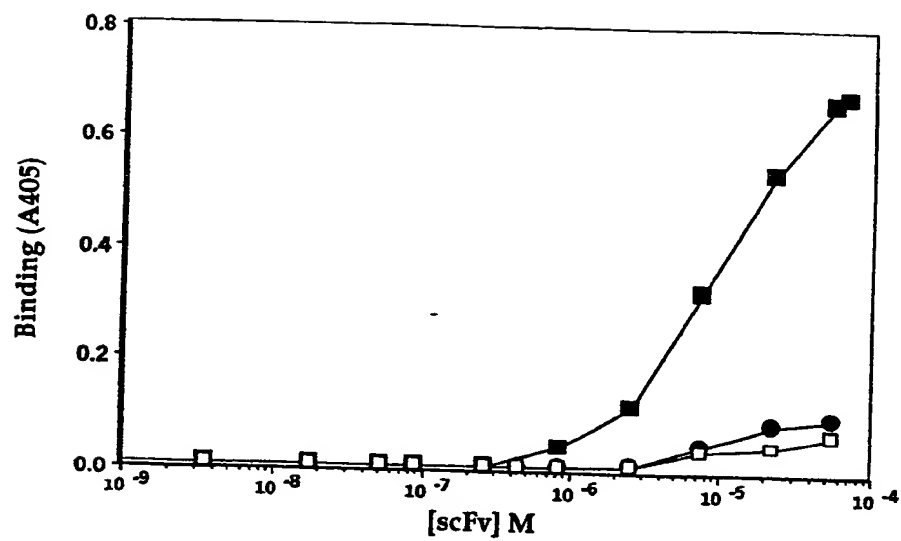


FIGURE 7

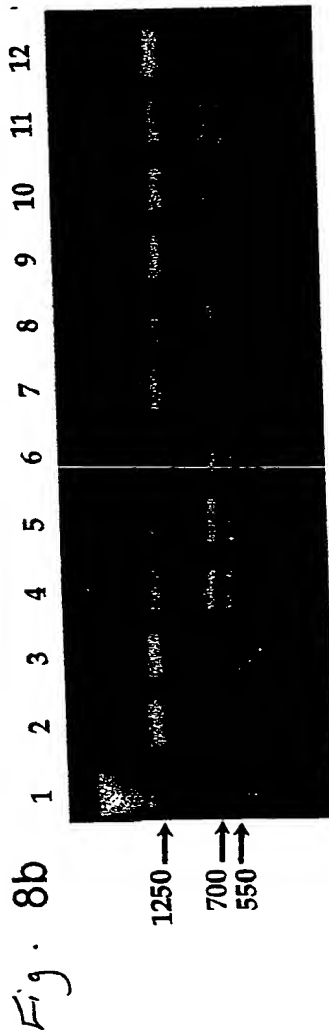
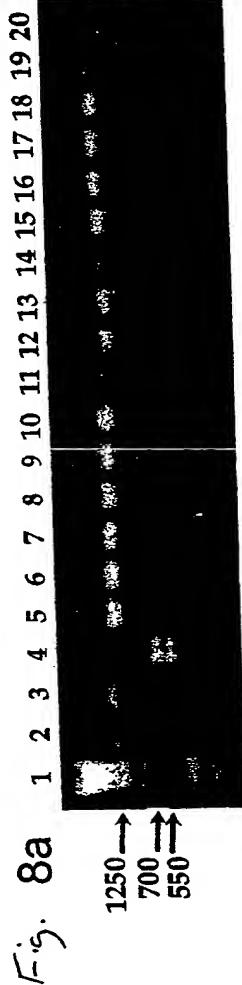


Fig. 9a

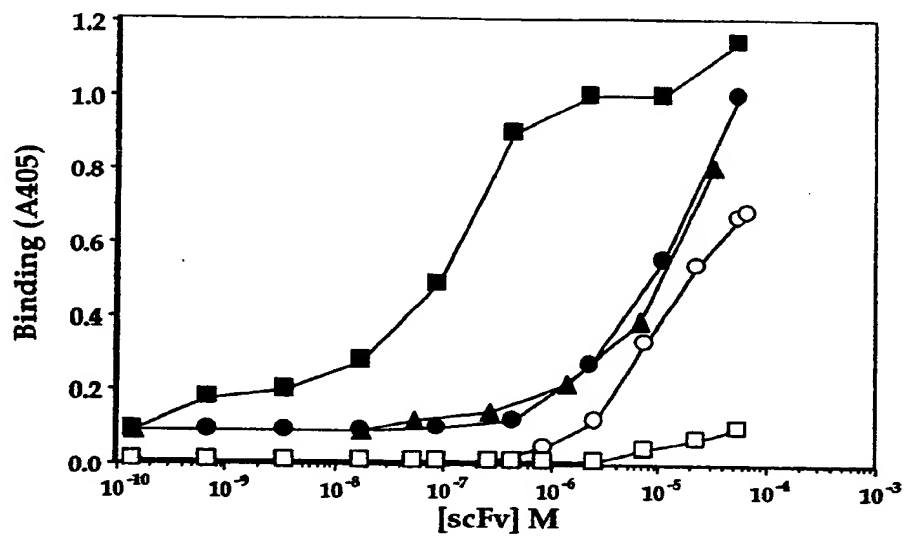
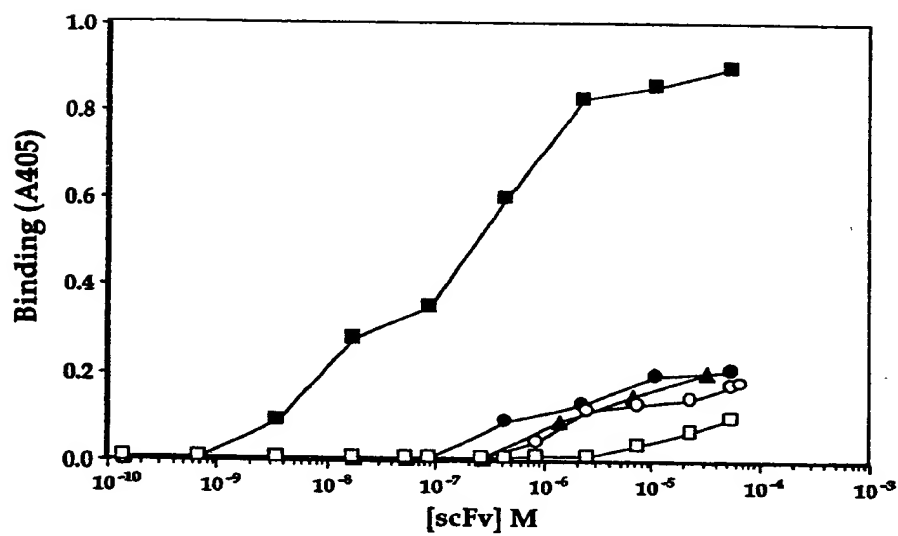


Fig. 9b



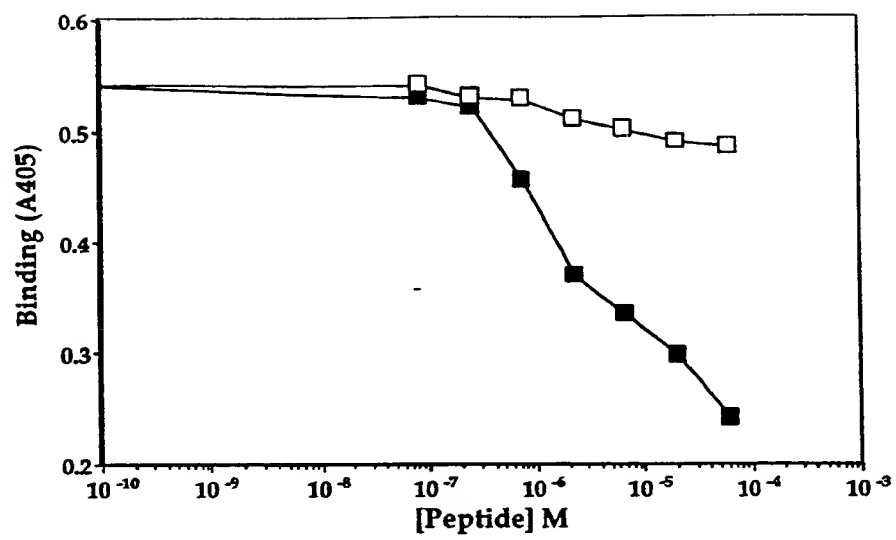


FIGURE 10

Fig. 11a.

CAG GTC AAA CTG CAG GAG TCA GGC GCT GAG CTG GTG AGG OCT GGG GTC TCA GTG AAG ATT
 gln val lys leu gln glu ser gly ala glu leu val arg pro gly val ser val lys ile
 TOC TGC AAG GGT TCT GGC TAC ACA TTC ACT GAT TAT GCT ATG CAC TGG GTG AAG CAG AGT
 ser cys lys gly ser gly tyr thr phe thr asp tyr ala met his trp val lys gln ser
 CDR 1
 CAT GCA AAG AGT CTA GAG TGG ATT GGA GTT ATT AGT ACT TAC TAT GGT GAT GCT AGC TAC
 his ala lys ser leu glu trp ile gly val ile ser thr tyr tyr gly asp ala ser tyr
 CDR 2
 AAC CAG AAG TTC AAG GGC AAG GGC ACA ATG ACT GTA GAC AAA TOC TOC AGC ACA GGC TAT
asn gln lys phe lys gly lys ala thr met thr val asp lys ser ser ser thr ala tyr
 CDR 2
 ATG GAA CTT GCC AGA CTG ACA TCT GAG GAT TCT GCC ATC TAT TAC TGT GCA AGA GGC GCT
 met glu leu ala arg leu thr ser glu asp ser ala ile tyr tyr cys ala arg gly ala
 CDR 3
 ACT ATG TOC TAC TTT GAC TAC TGG GGC CAA GTG ACC ACG GTC ACC GTC TOC TCA ggt gga
thr met ser tyr phe asp tyr trp gly gln val thr thr val thr val ser ser gly gly

Fig. 11b.

ggc ggt tca ggc gga gtt ggc tct ggc ggt ggc gga tgg GAC ATC GAG CTC ACT CAG TCT
gly gly ser gly gly val gly ser gly gly gly gly ser asp ile glu leu thr gln ser
 Linker
 CCA GCA ATC ATG TCT GCA TCT CCA GGC GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC TCA
 pro ala ile met ser ala ser pro gly glu lys val thr met thr cys ser ala ser ser
 CDR 1
 AGT ATA AGT TAC ATG CAC TGG TAT CAG CAG AAG CCA GGC ACC TOC CCC AAA AGA TGC ATT
ser ile ser tyr met his trp tyr gln gln lys pro gly thr ser pro lys arg trp ile
 CDR 1
 TAT GAC ACA TOC AAA CTG GCT TCT GCA GTC OCT GCT GGC TTC AGT GGC AGT GGC TCT GGC
 tyr asp thr ser lys leu ala ser gly val pro ala arg phe ser gly ser gly ser gly
 CDR 2
 ACC TCT TAT TCT CTC ACA ATC AGC AGC ATG GAG GCT GAA GAT OCT GGC ACT TAT TAC TGC
 thr ser tyr ser leu thr ile ser ser met glu ala glu asp ala ala thr tyr tyr cys
 CAT CAG GGC AGT AGT TAC CCA TTC ACG TTC GGA GGC GGC GGC AAG CTG GAA ATA AAA
his gln arg ser ser tyr pro phe thr phe gly gly gly ala lys leu glu ile lys
 CDR 3

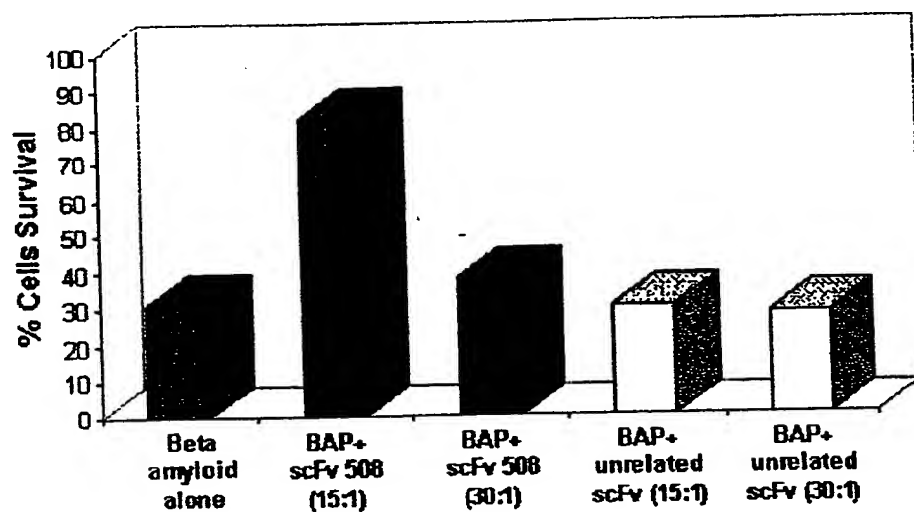


FIGURE 12

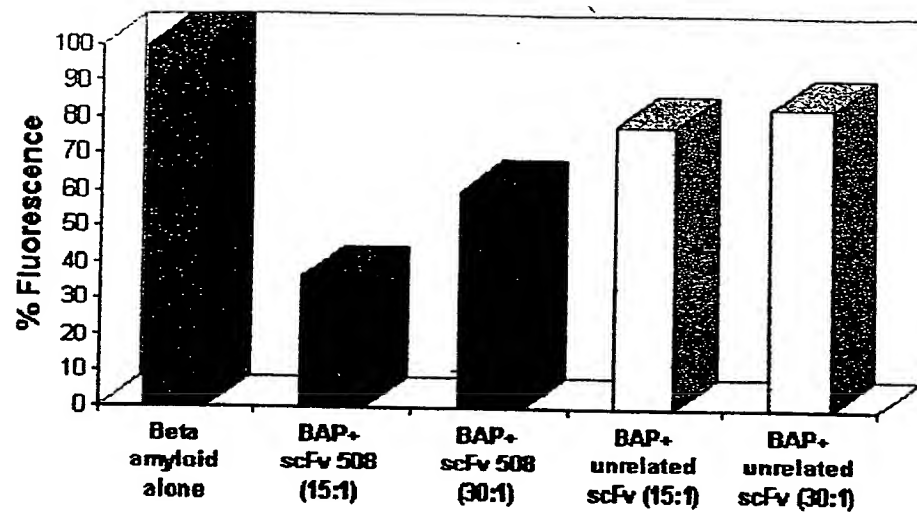


FIGURE 13

Fig. 14c



Fig. 14d



Fig. 14a



Fig. 14b

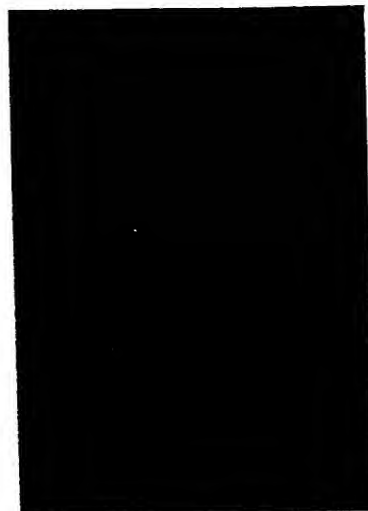


Fig 15c

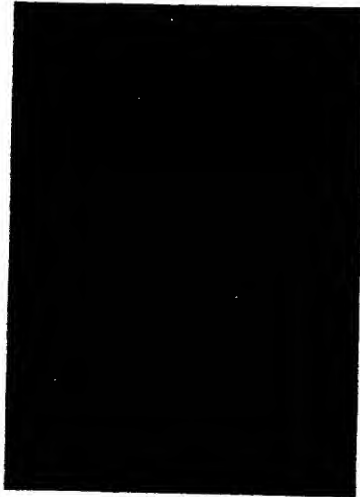


Fig 15d



Fig 15a

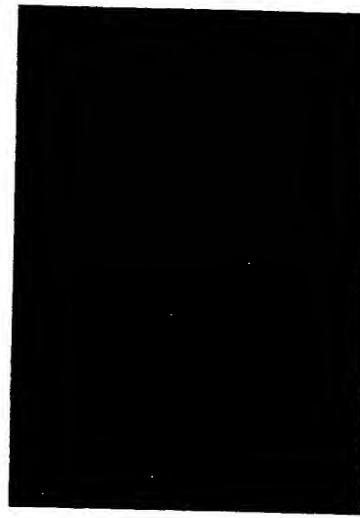


Fig 15b



SECRET

Fig. 16c



Fig. 16d

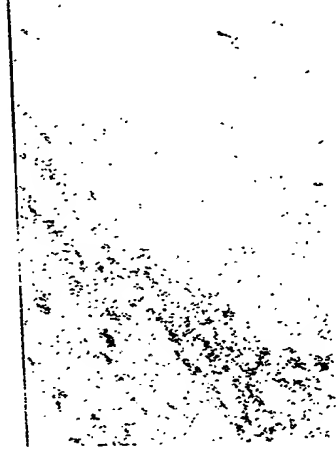


Fig. 16a



Fig. 16b



SECRET

Fig. 17a

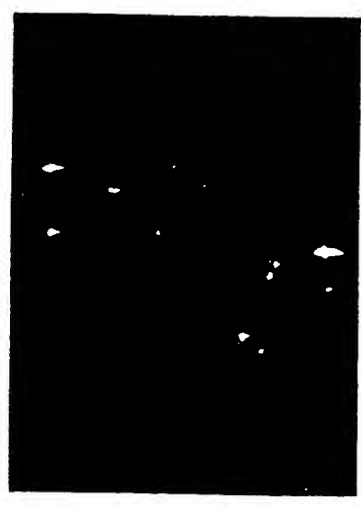


Fig. 17b



Fig. 17c

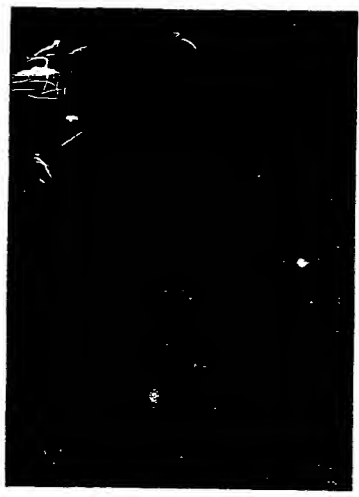
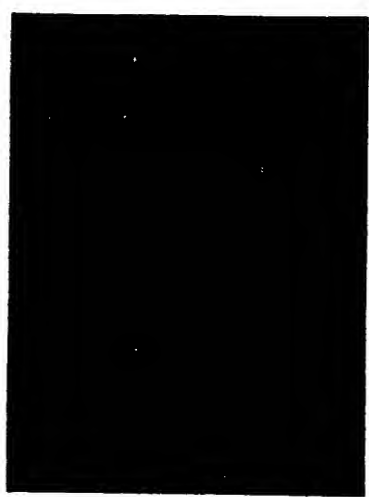


Fig. 17d



SECRET

Fig 18c



Fig 18d



Fig 18a

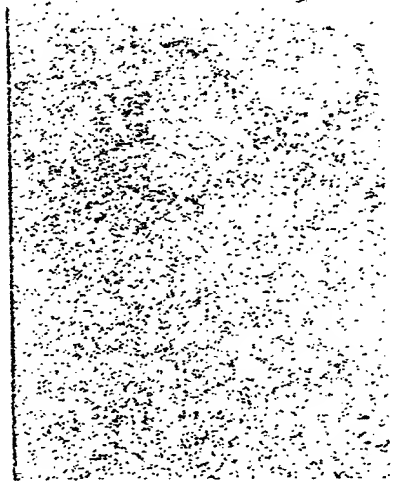
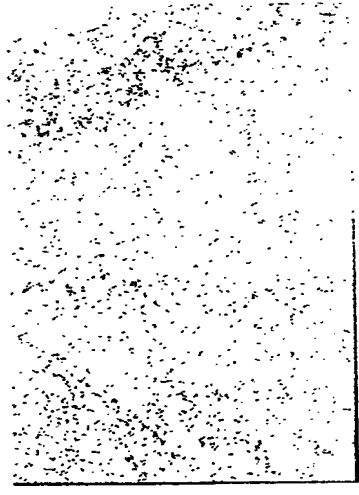


Fig 18b



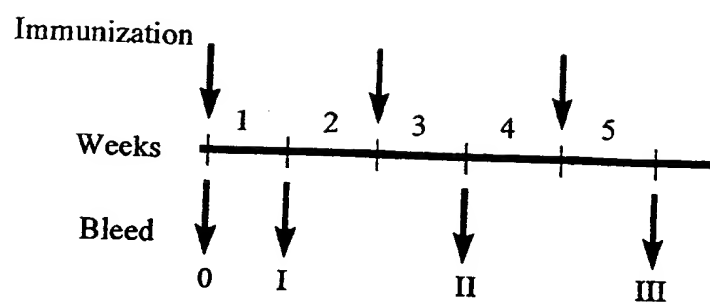


FIGURE 19

Fig. 20a

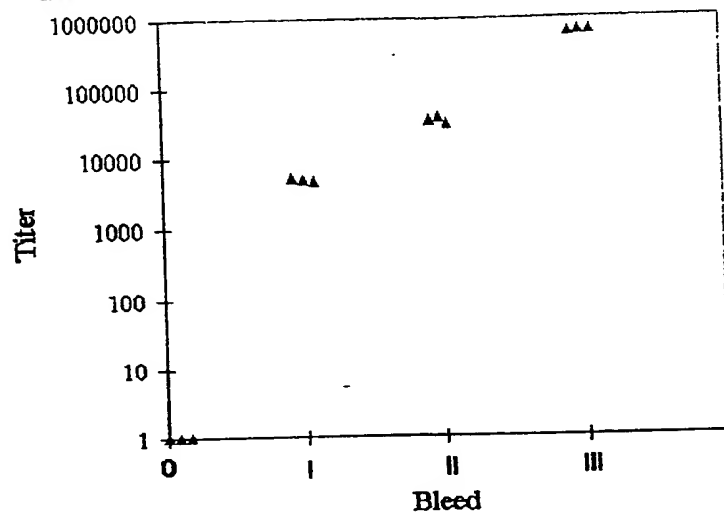
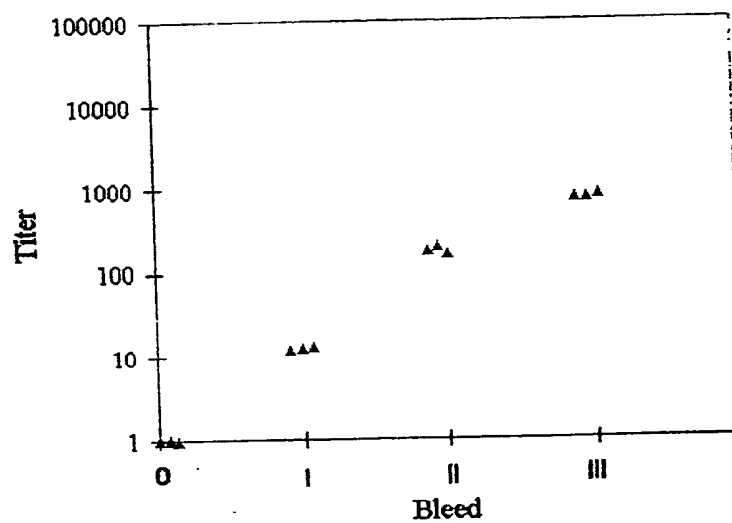


Fig. 20b



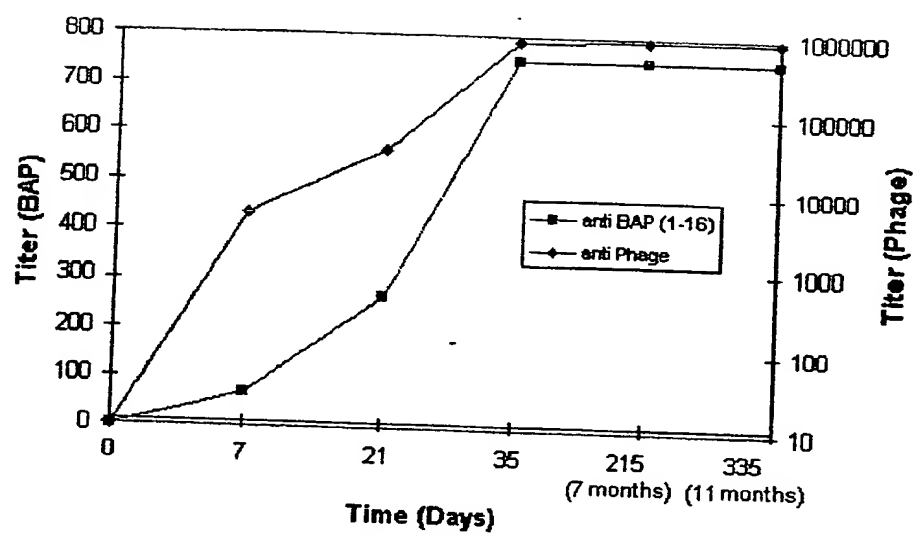


FIGURE 21

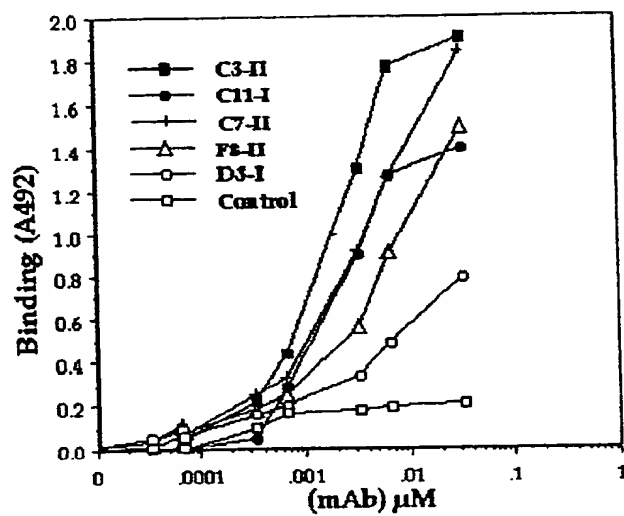


FIGURE 22

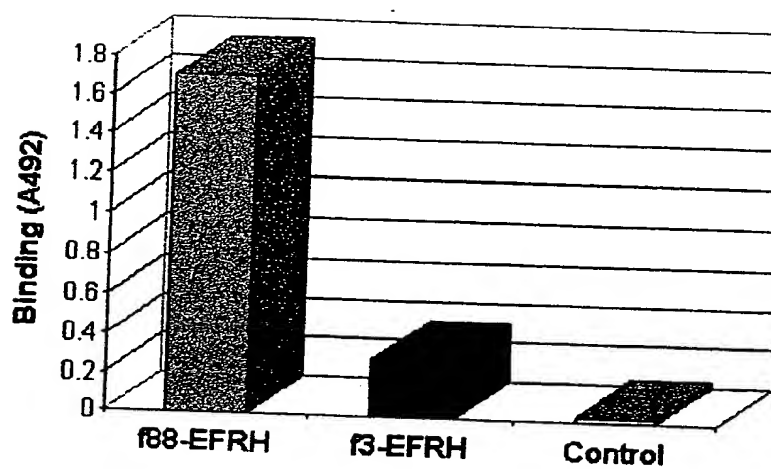
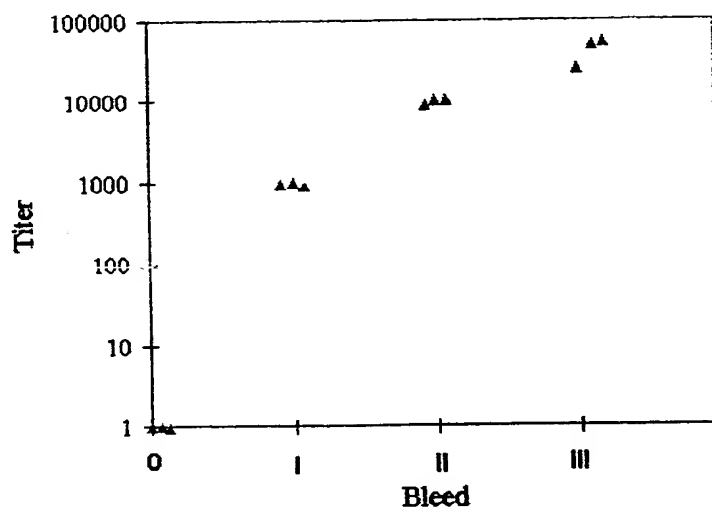
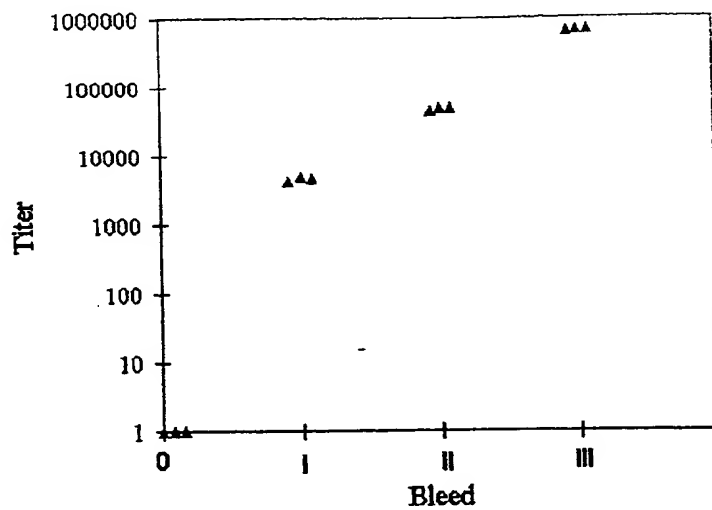


FIGURE 23



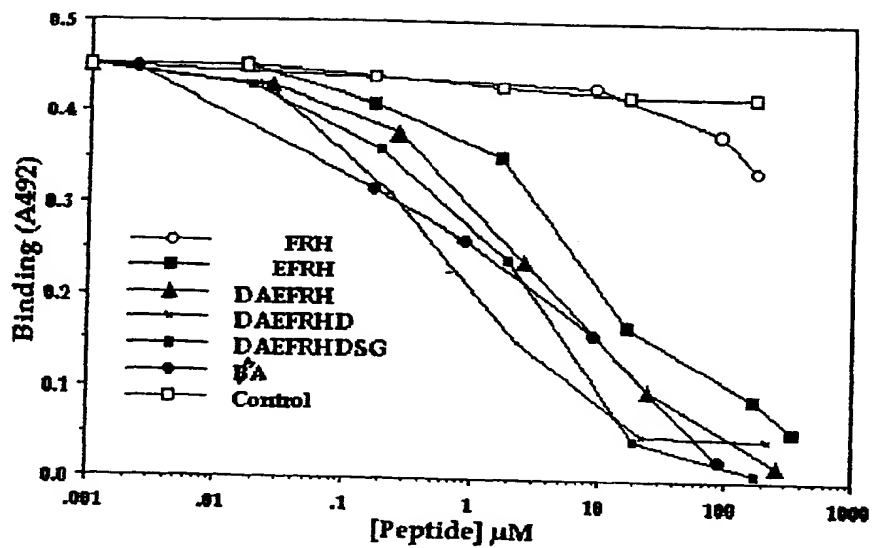


FIGURE 25

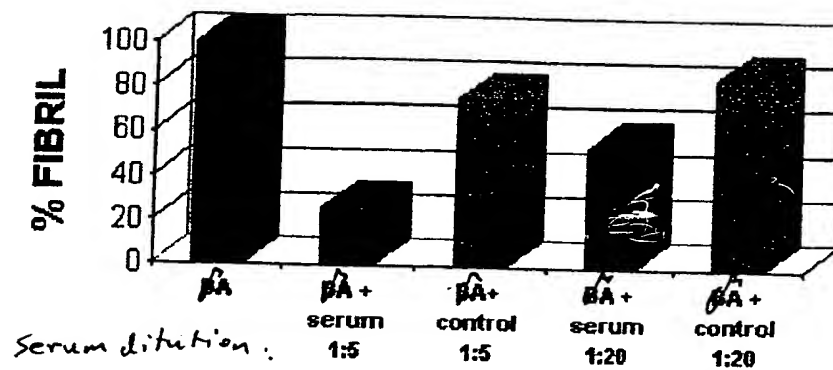


FIGURE 27

